

THE ACTION OF VITAL DYES IN TELEOSTS

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The demonstration, by Bouffard ('06) and by Goldmann ('09), showing that animal tissues may be stained *intra vitam* by the injection of solutions of certain benzidine dyes, quickly led to the recognition of the fact that the body possesses scattered groups of closely allied mononuclear cells, capable of absorbing and storing colloidal substances of all degrees of dispersion. These related cells, possessing the ability to ingest and house particulate matter, have been variously designated as macrophages, clasmatocytes, pyrrhol-cells, endothelial leucocytes and histiocytes.

The benzidine dye-stuffs, forming, as they do, ultra-microscopic solutions, many of which are practically nonirritant to living tissue, afford ideal conditions for the study of this class of cells and have served generally for their recognition. With a knowledge of the action of these dyes a reinvestigation of many normal and pathological processes has become necessary.

A comparative study of the distribution and fate of vital-dyes in the different groups of vertebrates, though important, has never been undertaken. Outside of mammalia, the behavior of vital-dyes has been little observed; this is due to the fact that mainly clinicians have been interested in the problem of vital-staining.

It is the object of the present paper to present some observations on the absorption, distribution and excretion of vital-dyes in the bony-fish, and in doing so, to furnish additional data whereby a comparative study of the cells affected by these substances may be facilitated.

There are a number of publications by Metchnikoff and his coworkers dealing with certain aspects of phagocytic activity in

fish. Metchnikoff ('05) was aware of the fact that red blood-corpuscles of the guinea-pig, when injected into the peritoneal cavity of gold-fish (*Cyprinus auratus*), were quickly seized upon and phagocytized by mononuclear cells inhabiting the serous cavity. His pupil, Mesnil ('95), while studying the reaction of teleosts (*Perca fluviatilis*, *Gobii fluviatilis*, *Carassius auratus*) towards injected virulent anthrax bacilli, demonstrated that the pathogenic organisms were ingested and rendered innocuous by numerous mononuclear cells. These elements Metchnikoff refers to as haemo-macrophages, averring that they are related to other groups of mononuclear phagocytes resident in the spleen, bone-marrow and lymph-nodes; and he describes them "as leucocytes with abundant protoplasm which stain readily by basic aniline dyes, whose nucleus, however, is sometimes divided into lobes." The author finds no other literature concerning the mononuclear phagocytes of fish.

The fish used for the following investigations were a species of carp, common to the fresh waters of the United States and originally introduced from Europe; they belong to the family Cyprinidae and genus *Carassius*, and, as nearly as could be determined, are closely allied or perhaps identical with *C. auratus*. The animals are an olivaceous color, their length averaging 15 centimeters, their weight 75 grams. They are extremely hardy and may be kept for months in the laboratory in aquaria supplied with running tap-water.

One cubic centimeter of a 1:100 solution of trypan-blue, was injected into the peritoneal cavity of each fish with as nearly sterile technique as possible. The whole procedure took less than a minute and the animals seemed absolutely undisturbed by it.

The first noticeable effect appears a few hours after the injection of the coloring matter, and consists in a gradually developing blue coloration of the integument, an indication of a rapid absorption of the dye from the peritoneal cavity.

The mode of absorption into the circulation from the peritoneal space appears to be by diffusion of the solution through the walls of the blood-vessels. Although there seems to be a slow

diffusion of the injected fluid through all the peritoneal surfaces, there is most probably a selective absorption by vascular tissue; for, if the peritoneal sac is opened twelve hours after introduction of the liquid, the blood-vessels are strikingly stained and contrast markedly with the pallor of the other tissues. The property of absorbing solutions is equally characteristic of arteries and veins of all calibres, and is easily demonstrated by preparing teased specimens of the fresh tissue. The dissolved dye seems to reach the circulation by passing directly through the cytoplasm of the elements of the vascular wall, as well as between the cells.

The omentum is practically undeveloped in pisces, and can play no rôle in the absorption of fluids from the peritoneal area as it does in higher vertebrates. From the study of smears, made at frequent intervals from the surface of the mesentery and peritoneal lining, it would appear that phagocytes play but little part in the primary peritoneal absorption of such colloids as trypan-blue, although a gradual concentration of the dye in large subperitoneal clasmatocytes takes place.

Inside of a few hours all of the body tissues are colored a pale blue, with but few important exceptions. The central nervous system never exhibits a blue coloration; nor does the fat, which is especially abundant in the mesentery of fish, show any staining.

Sections made of the tissues during the first two days reveal practically nothing except a diffuse blue staining, which in some instances is hardly visible under the microscope. Storage of the dye is somewhat more delayed than in mammalian tissue and its presence in definite cells is scarcely to be detected before the third day. Thenceforward its segregation to particular groups of cells becomes more and more striking, till, on the fifth or sixth day, the inclusion of the dye in the mononuclear phagocytes of the tissues is complete.

Specimens, in which the concentration of the dye had attained its greatest intensity, were fixed for the preparation of permanent sections. The tissues, after fixation in 10 per cent formalin, were dehydrated and cleared, imbedded in paraffin and cut at 5 microns. The sections were stained with Meyer's alum-carmine.

The liver, which, in Cyprinidae, is a relatively small organ, is completely concealed by the intestines on opening the peritoneal cavity. It consists of numerous loosely connected lobes, closely adherent to the portal veins and very friable, and in an animal which has been colored vitally, it is the most deeply stained tissue in the body. Microscopically the liver of fish neither presents an arrangement into lobules, as in higher animals, nor does it display the cell-tubes characteristic of amphibia. It consists of strands of cells laid down according to no definite pattern. These are abundantly vascularized from the hepatic portal veins and the hepatic arteries which break up into a number of sinusoid spaces. The sinuses are lined by vitally stained endothelial cells, which in appearance and behavior correspond in every respect to the cells of Kupffer in the higher forms. The vital-dye is densely aggregated in the Kupffer cells, whereas the hepatic cells do not contain particles of stain, although they so often do in other animals. The author is able to affirm, however, that the absence of dye-granules from the hepatic parenchyma is not a fixed rule in fish, since the livers of dog-fish (*Mustelus canis*) which were similarly stained, showed both hepatic and Kupffer cells containing the dye.

The Kupffer cells of ichthyopsida appear to be relatively more numerous than in mammalia; they possess the same power of amoeboid movement and they are frequently seen partially detached from the wall of a sinus or lying perfectly free within one, as round, vitally stained mononuclear cells.

The gall-bladder and bile-ducts show no cellular staining of any description, although demonstrable traces of dye are present in the bile. The color seems to gain access to the bile as a result of direct diffusion through the duct-walls.

The spleen, in the species studied, forms a series of dark red partially connected bodies which are distributed along the entire course of the intestine, and are intimately associated with the lobes of the pancreas. The splenic tissue in Cyprinidae, as in fish generally, belongs to the category of haemolymph-glands. On section, such a nodule reveals a delicate capsule, and a framework of slender trabeculae dipping into the depths of the gland,

which divide it into a labyrinth of spacious vascular sinuses. Two things strike one at once,—the great vascularity of the sinuses, which are densely packed with oval, nucleated red blood corpuscles, and the relatively small amount of spleen pulp, containing but few lymphocytes.

The vascular sinuses are partially lined by endothelial cells which are actively phagocytic towards trypan-blue, which phenomenon allies them with the reticulo-endothelium seen in the mammalian spleen. The reticulo-endothelial tissue in fish is extremely simple, for one meets everywhere only an incomplete layer of vitally stained endothelial cells which line the vascular spaces, whereas more highly developed spleens possess a complicated network of phagocytic reticulum cells. The fact that, one does not find endothelial cells in the act of desquamating, nor free phagocytes within the sinuses as one does in mammals, may be a sign that the fish's spleen is only poorly adapted for phagocytic activity and represents a more primitive stage of development.

The remaining organs of the peritoneal cavity, with the exception of the Wolffian body, may be reviewed quickly, for they showed nothing striking from the standpoint of vital-staining. The pancreas, and the elements of the gonads, both testis and ovary, are practically unstained. The intestines exhibit but a diffuse blue coloring of the serosa and the muscular coats; the contents of the canal are colorless.

The Wolffian body (mesonephros) of the species of Cyprinidae studied, is a flattened, elongated organ, closely applied to the dorsal wall of the coelomic cavity. It stains very deeply with vital-dyes, and presents, on gross inspection, in an animal stained with trypan-blue, a uniform deep blue color. The excretion of the benzidine dyes through the mesonephros commences very quickly after their initial introduction into the coelom.

Trypan-blue, as von Möllendorff ('15) showed by dialysis experiments, is in fact a mixture of two dyes. One of these, a red substance, is very diffusible, the other, a blue coloring matter, passes through semi-permeable membranes only very slowly and under ordinary circumstances masks the red element.

These two constituents are readily demonstrated by putting a drop of trypan-blue upon a piece of filter-paper, whereupon a red zone surrounding a blue center immediately forms. Von Möllendorff actually observed the difference in the rate of diffusion of these two substances in the mammalian kidney by obtaining specimens of urine at frequent intervals. He noticed that the earliest specimens collected were a pink color, whereas the subsequent ones turned to purple and finally to blue.

If a fresh preparation is made, of a bit of tissue from the Wolffian body of a fish eighteen hours after intraperitoneal injection of trypan-blue, the tubular epithelium is seen to be tinged red, while a blue coloration is visible only in the interstitial tissue of the kidney. The assumption is that the tubular epithelium is readily permeable to the pink, but to a slight extent only to the slowly diffusible blue constituent of the dye. After a week or more, the red substance becomes exhausted, the pink coloration of the tubules disappears, and they become colorless, excepting some which exhibit traces of blue. It will be shown, that in the mesonephros of teleosts the diffusion of the blue substance to any extent into the tubular parenchyma and so out into the urine, is prevented by a mechanism presently to be described.

The blood supply of the mesonephros of fish resembles very closely that of the liver. The caudal vein bifurcates close to the cloaca, forming two venous trunks which in turn give off a number of advehent vessels to the Wolffian body. These soon break up into a capillary plexus or rete between the tubules, and finally reunite to open by revehent channels into the posterior cardinal veins. This venous capillary-bed with its afferent and efferent vessels is spoken of as the renal portal system and corresponds in every sense to the hepatic portal system familiar to everyone. The mesonephros derives its arterial blood from branches of the aorta through vessels analogous to the hepatic arteries, on which the glomeruli are formed.

The blue component of trypan-blue with which we are actually dealing in vital-staining, gradually accumulates in the intertubular tissue of the Wolffian body. If at the height of

staining, thin paraffin sections are prepared, one is struck by the remarkable distribution of the accumulated dye. The tubules, as noted in the fresh preparations, are nearly dye-free. The accumulations of trypan-blue in the intertubular substance however, are found in the endothelium of the renal portal system, so that one gazes upon a rich network of brilliantly stained capillaries. The endothelial cells lining them appear to possess a great avidity for benzidine dyes, judging from the enormous concentration of coloring matter within their cytoplasm. The glomerular endothelium, which embryologically is a derivative from the aorta, remains unstained.

The stain occurs also, as has been already noted, as blue, finely granular deposits in the distal cytoplasm of a few of the epithelial cells of the renal tubules, in no way comparable however, to the large masses of dye stored in the adjacent vascular endothelium. The epithelium does not in any way resemble, in its behavior towards vital-dyes, the convoluted tubules of the metanephros, whose living cells are always densely crowded with dye. The cells lining Bowman's capsule are unstained.

It is important to recall that in the liver, where similar vascular conditions prevail, we have a corresponding phagocytic activity developed by endothelium—the Kupffer cells—and that at times the liver parenchyma itself contains vitally stained particles. It seems reasonable to suppose that the phagocytic endothelial cells, observed in the mesonephros of teleosts, are analogous to the Kupffer cells in the liver, and that they possess two or possibly three valuable functions. The first function would be to prevent the excretion and consequent loss to the body, of colloidal metabolites circulating in the blood stream; such a conservation of organic nitrogen would possibly account for the relatively low nitrogen output determined for teleost fish by Denis ('13). The second function might well be a protective one, whereby toxic substances in the circulation, which could easily become injurious to the kidney-parenchyma and impair its functional activity, are rendered innocuous. Such a function would explain the retention of the trypan-blue by the endothelial cells of the renal portal system, and its non-appearance

in the tubular epithelium. Lastly they may be an important factor in the process of reabsorption of substances by the tubules, for, as Bainbridge, Collins and Menzies ('13) have shown in the kidney of the frog, one inorganic substance at least, sodium-chloride, is partially reabsorbed from the glomerular filtrate as it passes down the tubule. The fact that fine dye-granules appear in some of the tubules in spite of the phagocytic activity described for the renal endothelium, might be explained either as the result of reabsorption of trypan-blue from the glomerular dialysate, or, as seems more probable, as an excess which has escaped into the epithelial cells in spite of the barrier provided against particulate matter by the lining endothelium of the renal portal capillaries.

With the exception of the specialized vascular linings seen in the liver, kidney and spleen, the endothelium of the blood-vessels does not stain vitally. The outer coats of both arteries and veins, on the other hand, stain quite deeply, due to the presence of diffuse color in the elastic-tissue of the media and adventitia. For the same reason the bulbous arteriosus appears a dark blue, in striking contrast to the unstained myo- and pericardium of the ventricle. The red blood-corpuscles never exhibit vital-staining, whereas an occasional polymorphonuclear or mononuclear leucocyte in the circulation may show a blue coloration. Such vitally stained mononuclear cells probably escape in small numbers into the systemic circulation from the liver sinuses.

The lymphatic endothelium in Cyprinidae is capable of absorbing and storing vital-dyes, a fact of particular interest which is most easily demonstrated in the lymphatic plexus which arises in the corium of the species studied. If the scales are removed from a vitally stained fish, and a small film of the exposed corium dissected free and placed upon a slide in a drop of glycerin, or stained with alum-carmin and mounted in balsam, and examined, an abundant network of vessels of varying dimensions is visible. Part of these vessels resolve themselves into a lymphatic plexus, whose endothelial lining-cells contain dark blue deposits of granular dye within their cytoplasm, a phenomenon which makes them stand out sharply as

a system of anastomosing channels which are readily distinguishable from blood-vessels. The blood vascular tree contains erythrocytes in many of its branches; one usually finds artery and vein accompanying one another, and it is possible to see blood-vessels crossing the vitally stained canals without anastomosing with them—definite proof of the independence of the two sets of vessels. The blood vascular lining-cells never contain even so much as a trace of vital-dye; and in fact, in fish, trypan-blue has never been observed to occur in the endothelium of any blood-vessels other than the renal and hepatic portal plexuses.

Distally the lymphatics of the corium arborize about the scale-pouches, while proximally they are traceable into large, easily collapsible, thin-walled vessels, whose endothelium is similarly phagocytic towards trypan-blue. These vessels follow a course in the intermuscular septa and finally empty into dorsal and lateral longitudinal collecting channels.

The author has examined preparations from every part of the body in the same way, and has been able to ascertain the presence of lymphatics in nearly all connective tissue. Deeply stained lymphatic sinuses with numerous branches were noticed accompanying the aorta. The course of many heavily stained lymphatics was noted in the septa and membranes of the serous cavities, besides a rich anastomosis of vessels in the subperitoneal tissues. In every instance the lymphatic endothelium shows the same phagocytic behavior towards vital-dyes as that seen in the corium.

The observation that the endothelium of the lymphatic vessels of Cyprinidae may be stained by vital-dyes appears to be of considerable importance. It affords an ideal means, combined with an injection technique such as Allen ('06) used, for studying the distribution of lymphatics in the adult animal, and furthermore it promises to lend itself to a solution of the question of the origin and development of the lymphatic system in fish. Favaro ('06) and Mozejko ('14) feel, from their studies, that the lymphatics of fish arise, by a direct transformation of veins into lymphatic vessels, at a late stage of development; so that they constitute modified veins rather than true lymphatics, in

the sense of outgrowths from the embryonic veins, as in higher vertebrates. McClure ('14) on the other hand believes that he has demonstrated the formation of lymphatics in trout embryos quite independent of the veins, as a series of separated vesicles whose endothelium is of mesenchymal origin. The author's observations would tend to show that the lymphatics of fish are not modified veins in the sense of Mozejko, for as has been stated above, a fundamental difference exists between the endothelium of vein and lymphatic in their behavior towards vital-dyes, which makes it improbable that a sudden transformation from one into the other should occur.

On the other hand it seems unlikely that lymphatics in fish should form from tissue-spaces by the transformation of mesenchyme cells into endothelium instead of from embryonic veins, because nowhere else in vertebrates have lymphatics been proven to arise in such a manner. Clark ('09) demonstrated that in the tadpole lymphatics grow by a process of budding and sprouting, and that lymphatic endothelium is always formed from preexisting endothelial cells, but never from mesenchyme. The author ('16) confirmed Clark's work and showed further that the endothelium of the lymphatic channels of tadpoles is phagocytic towards vital-dyes, an observation which affords a simple method of distinguishing endothelium from mesenchyme cells. From the present report it is apparent that the lymphatic endothelium of teleosts and of amphibian larvae behave just alike towards benzidine dyes; and it does not seem unreasonable to infer that, not only their morphology, but their growth and development are the same.

A last point of interest is that, in fish and amphibian larvae, there is a primitive lymphatic system whose entire endothelium possesses a function, which, in higher vertebrates, is consigned entirely to the reticulo-endothelial cells of specialized parts of the lymphatic apparatus, the lymph-glands. It is difficult to say what the advantage could be of such a specialization in the distribution of nutritional and phagocytic activity (for the storage of benzidine dyes in the cell may be undoubtedly interpreted in these terms), in higher animals.

The 'wandering cells' or clasmatoocytes of fish need no special description for they resemble in every way those of mammalia, although they are not nearly so numerous, and, in fact, there are very few in the subcutaneous tissue and myotomes. They occur abundantly in the membranes of the serous cavities, where they may be seen as 'adventitial cells' in close proximity to blood-vessels. Smears from the peritoneal fluid also reveal the presence of free macrophages in the body-cavity. The mesothelial cells lining the coelomic sac are, however, non-phagocytic.

The origin of the phagocytic mononuclear cells in the serous cavities is as obscure in fish as it is in higher vertebrates, the supposition being that they are derived from histogenous lymphocytes or clasmatoocytes.

The remaining tissues need little description, though the integument requires brief attention. The scales of *Carrasius auratus* are relatively large. Microscopically they exhibit numerous concentric rings or annuli, transected by eight to twelve radii, which diverge from the focus and end at the periphery of the scale. The focus appears to be formed by the fusion of a number of polygonal plates, between which the sutures are plainly visible. In an animal stained *intra vitam*, with trypan-blue, the dye appears in the scales an hour or so after introduction of the substance, a circumstance which accounts for the rapidity with which the animals assume a dusky blue hue. The distribution of the dye in the scale is very characteristic. The sutures at the focus of the scale are colored, and the radii injected with a deep blue stain from the center to the margin of the scale, the whole presenting the appearance of a network with eight or more blue spokes radiating from it. The annuli are unstained. The inference is, that the scale possesses a system of canals or spaces, in close association with the circulation, through which it is possible for nutritive substances from the blood-stream to reach to all its parts. These spaces, if they are actually in connection with the external surface of the scale, might possibly serve as an excretory apparatus.

The central nervous system is unstained, remaining perfectly white throughout, a contrast to the other tissues of the body,

which, on gross inspection, all present a diffuse blue coloration. The behavior of the nervous tissue coincides exactly with the findings in higher animals.

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SUMMARY

Fish are readily stained *intra vitam* by injecting weak solutions of benzidine dyes into the peritoneal cavity. Absorption of the dye from the coelom, by way of the blood-vessels into the circulation, and diffusion throughout the body occurs within a few hours.

Concentration of the dye within the cytoplasmic vacuoles of the 'makrophages' of the tissues occurs only very slowly, so that a striking segregation of coloring matter is not demonstrable before the end of a week.

The endothelium of specialized tracts of the vascular and lymphatic apparatus plays an important rôle in the binding and storage of the dye in teleosts.

It may be observed that benzidine dyes are phagocytised by the endothelium of the hepatic sinuses, analogous to the Kupffer-cells in mammals, and by the reticulo-endothelium of the spleen, which however is much less extensive in character in fish than in higher forms.

In addition to the appearance of dye in the liver and spleen, one notices a storage of vital-stain in the endothelium of the renal portal system, a phenomenon which has no counterpart in the metanephros, nor does it occur in the Wolffian body of amphibia.

There is furthermore a widespread storage of vital-dye on the part of the endothelium of the lymphatic vessels, a condition also noted in amphibian larvae, but never known to occur in mammals.

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REACTIONS OF BLOOD- AND TISSUE CELLS TO ACID COLLOIDAL DYES UNDER EXPERIMENTAL CONDITIONS¹

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FOUR FIGURES (ONE PLATE)

In recent years the employment of various colloidal suspensions as a method of intra vitam 'staining' has come into such general usage that it is hardly necessary to give a general review of the literature concerned or to describe the details of the technique. Collargol, lithium carmine, and the colloidal acid azo dyes Trypanblau, Pyrrholblau, etc., are the substances which have been most commonly used, with little or no variation in the final results. Since these substances are practically non-toxic for mammals it is possible to inject the same animal with repeated large doses of aqueous suspensions before he shows any ill effects from the treatment.

The dyes usually appear in the cells in the form of granules, diffuse staining of the cytoplasm and nucleus probably always being an indication of injury of the cell. Since the dyes which have generally been employed diffuse rapidly through the tissues, it makes little difference whether the animal has been stained by intravenous, intraperitoneal or subcutaneous injections. The first two methods are preferred, because tissue necrosis at the site of injection is apt to result from repeated subcutaneous injections unless the parts are carefully massaged and kneaded.

Evans and Schulemann have shown that the rate of diffusion depends on the size of the particles of the dye (acid azo dyes) when in aqueous suspension. The more rapid diffusibility and

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the lessened danger from embolism would, therefore, make the dyes having the smaller particles the more favorable ones for experimental purposes. The rate of diffusion of a given dye also varies somewhat with the different species of animals used, the writer having found that Pyrrholblau and lithium carmine will diffuse much more rapidly through the tissues of a wild rat than through those of a rabbit or guinea pig following subcutaneous injections of the dyes.

The 'dye granules', as they have been termed by Evans and Schulemann, have been reported in many different types of cells belonging to many different tissues, and, as is to be expected, the character of the granules varies considerably in the different types of cells. It is possible that some of these granules may represent instances of true vital staining of preformed structures, as has been claimed for all of the dye granules by several authors, and that others are mere aggregations of dye particles.

Schulemann ('12) believed that the dye granules represent a combination of dye with a reaction body of the cell, and also that various preformed structures, such as plasmosomes, secretory granules, etc., can be made visible by means of the dyes. V. Möllendorf, Pappenheim and Tschaschin also agree with this earlier view of Schulemann. According to Tschaschin the fine granules and rods seen in fibroblasts after Pyrrholblau injection, and the small blue granules of clasmatoocytes are chondriosomes, while the larger round granules are secretory granules derived from the chondriosomes. K. J. Scott, however, has shown that the chondriosomes of these cells may be stained with Janus green while the cells contain the dye granules. She could find no relation between the latter and the chondriosomes.

At present Schulemann and Evans believe that the dye granules are merely aggregations of dye particles which have been taken up by a process similar to phagocytosis and deposited within cytoplasmic vacuoles without combining with any constituents of the cell. In other words, the formation of dye granules is a physical process, and not a chemical union of preformed structures or receptors of the cell with the dye. In his

latest paper Schulemann ('16) claims to have demonstrated the physical nature of the granules experimentally.

Although dye granules may be found in many cells belonging to very different tissues, there is one group of closely related cells which shows special avidity for the dyes. With repeated injections these cells will store more and more of the dye in the form of very coarse irregular 'dye granules'. Cells belonging to this group are the so-called clasmatocytes or resting wandering cells of the loose connective tissue, the fixed cells of the reticulum of the hematopoietic organs, especially those which line the sinuses of lymph nodes, and the free cells derived from the reticulum, the v. Kupffer 'stellate' cells of the liver, and the non-granular lymphoid cells of the milky patches of the omentum. The free lymphoid cells of the peritoneal cavity also seem to belong in the group, since many of them show the same reaction after intraperitoneal injections of the dyes. Numerous histological studies by Maximow, Weidenreich, Pappenheim, Downey, and many others have established the intimate relationships between the different members of the group, and to a more or less extent these relationships are now generally recognized, so it is hardly necessary to undertake the extensive review of the literature which would be required in order to point out the details.

Various names have been applied to these cells by those who have studied them by means of the colloidal dyes. They are the pyrrhol cells of Goldmann, the histiocytes of Aschoff-Kiyono, the resting wandering cells of Tschaschin, and the macrophages of Evans. The grouping of all these different types of cells under one term indicates the general belief in their close relationship. Because they are the cells which are especially concerned in the reactions to the colloidal dyes it has been assumed by most workers in this field that the reaction is absolutely specific, i.e., that it always enables us to distinguish clearly between the members of this group and other cells which do not belong to the group, more especially those of the blood.

That the lymphoid cells of various types should be divided into a group which is made up of cells which are primarily blood

cells, and into another group which is of tissue origin, has been claimed by many, particularly by Pappenheim. This idea of the dual nature of the lymphocytes has even been carried over to the lymphoid organs. Here it is supposed that the cells of the spleen pulp and of the interfollicular tissue of the lymph nodes are different in origin and developmental potentialities from those of the follicles. The fact that many anatomists have demonstrated the radial migration of the lymphocytes into the pulp and interfollicular tissue has been completely ignored by those who have accepted this theory.

One of the chief difficulties which the above theory has encountered has been the fact that it is impossible to demonstrate any morphological differences between the lymphoid cells of the tissues and those of the blood and blood-forming organs. However, the solution of this difficulty seemed accomplished with the advent of the methods of vital staining with colloidal dyes, for it was found that intravenous injections of large quantities of the dyes did not result in the staining of any of the blood cells, while all of the cells of the tissues belonging to the group of histiocytes were intensely stained. This led Aschoff-Kiyono, Pappenheim, Schulemann-Evans and others to the belief that the 'dye cells' were always of tissue origin, and this in spite of the fact that Kiyono, one of the most ardent defenders of the specificity of the reaction, was forced to admit that lymphocytes of lymph nodes in the later stages of aseptic inflammation may enlarge and develop relatively more protoplasm, when it is impossible to distinguish them from the true histiocytes of the organ, and impossible to show that they do not take up the dye. Kiyono also admits that in the *tâches laiteuses* of the omentum of vitally stained animals it is difficult to distinguish between the smaller 'histiocytes' containing few granules and the larger lymphocytes. But in spite of this admission of the occurrence of intermediate forms Kiyono concludes that the method is a reliable one, and that the lymphoid cells of the tissues which take up the dye belong to the group of histiocytes rather than to the lymphocytes. To what extent this opinion has gained ground is shown by the recent publications of H. M. Evans and

Frank A. Evans. The latter, in an experimental study of the mononuclears of the blood and tissues, divides these cells into myeloid cells which show the oxydase reaction, macrophages which contain carmine granules, and lymphocytes which do not respond to either of these reactions. The supposed specificity of these two reactions is, therefore, accepted and the lymphoid cells are consequently divided into three distinct groups. According to this classification the bulk of the free cells of the spleen pulp are 'myeloid' cells, and the so-called transitional cells of the blood are also of myeloid origin. The cells with dye granules belong to the group of macrophages which are of tissue origin, while the free wandering cells without dye granules belong to the series of lymphocytes which are derived from the parenchyme of the lymphoid organs.

While the theory of the absolute independence of the histiocytes has been the commonly accepted one it has not passed without opposition. For theoretical reasons it is opposed by Pappenheim ('13) and by Emmel ('16, p. 106-108), both believing that the ability to take up the colloidal dyes is dependent on the stage of differentiation of the cells rather than upon complete genetic independence from other cells which do not ingest the dyes. Pappenheim is inclined to the belief that the histiocytes may be the mother cells of non-granular lymphoid cells which have lost the ability to take up the dyes during their further differentiation. Tschaschin, on the other hand, concludes from extensive studies with experimental inflammation of the loose connective tissue of vitally stained animals, that lymphocytes leave the vessels in great numbers and increase rapidly in size to form 'polyblasts' which take up the dye and store it in the form of the typical dye granules of the histiocytes. From this he reasons that there is no fundamental distinction between histiocytes and lymphocytes, but that the latter must leave the blood or lymph stream before they can take up the dye. Those of the hematopoietic organs or blood and lymph stream are too young and not sufficiently differentiated, or they are not in the proper medium, so they must leave the vessels before they are able to perform their special functions. According to Tschas-

chin then, blood cells and wandering cells of the connective tissue are closely related cells, and in many cases they are the same cells under different conditions and in different stages of differentiation.

Maximow arrived at similar conclusions from a study of tissue cultures of lymph nodes from young and adult rabbits. In such cultures he found that lymphocytes migrate into the plasma where they survive three or four transplantations. The addition of tissue extracts to the plasma prolongs the life of the lymphocytes and enables them to differentiate into typical polyblasts which will take up Trypanblau from the plasma and store it in the form of dye granules. Later these polyblasts cannot be distinguished from those which come from the reticulum. All the intermediate stages are traced and figured, and there seems to be no doubt in this case but what many of the cells which ingested the dye were derived from true lymphocytes of the lymph nodes. This is in harmony with Kiyono's observation that in the later stages of aseptic inflammation of lymph nodes it is impossible to distinguish between the larger lymphocytes and the smaller histiocytes. Kiyono, however, doubts the actual transition from one cell-form to the other.

It is generally conceded that under pathologic conditions, and following the stimulation resulting from repeated injections of the colloidal dyes, the reticular histiocytes from the hematopoietic organs, particularly the spleen, and the stellate cells from the liver may pass into the venous blood where they appear as large mononuclear cells loaded with dye granules. Few, however, are willing to follow Aschoff-Kiyono to the extent of believing that histiocytes form a small but constant percentage of the large mononuclears of normal blood. Although admitting the presence of histiocytes in normal blood Kiyono draws a sharp line between them and the lymphocytes and ordinary mononuclears without dye granules. The histiocytes are derived from fixed 'histioblasts' of the tissues and hematopoietic organs and their number is increased by proliferation of their own kind, while the lymphocytes are derived from the lymphoid parenchyme of the lymphoid organs.

The histiocytes do not take up the dye directly from the circulation but become loaded with it while they are still within the tissues, and their passage into the blood stream is more or less accidental, and is determined to a large extent by the conditions of the experiment. They are difficult to find in blood smears, but, as Aschoff and Kiyono have demonstrated, if the splenic, hepatic, or portal vein be tied off, fixed, embedded and sectioned they may be found in relatively large numbers. The fact that this procedure is necessary might be regarded as favoring the view of Mitamura and Masanori that the anesthetic, or the manipulation during the operation, or premortal agony cause the separation of the endothelial histiocytes from the hematopoietic organs and their entrance into the blood stream. These authors find the histiocytes to be very rare in the veins of living animals, but quite numerous in dead animals.

The view that their occurrence in the blood stream is more or less accidental is strengthened further by the observation of Aschoff-Kiyono that they are rapidly filtered out of the circulation by the capillaries of the lung, the vascular loops of the kidney glomeruli, etc. This shows that the dye cells act as foreign bodies when they reach the blood stream, and it also accounts for the small numbers of histiocytes in the general circulation.

Without further consideration the conclusion that the reaction to colloidal suspensions is a specific one might seem justified. However, Evans and Schulemann have shown that the process of taking up the dyes is really one of ingestion or phagocytosis rather than of true vital staining. If this is true we should naturally expect the reaction to conform to the general laws of phagocytosis. A few references to the literature will be sufficient to indicate a few facts regarding phagocytosis which are of importance in this problem.

While it is true that under certain conditions phagocytosis may take place within the general circulation this is not the rule, as can readily be proven by a few experiments with intravenous injections of foreign matter of various kinds. Recently Rosenthal has again shown that even living organisms are rarely phagocytosed within the circulating blood. Nonvirulent bacteria in-

jected into the tail vein of mouse were phagocytosed by 'endothelial' cells, chiefly the stellate cells of the liver. Wandering cells of the blood became active only when the bacteria were so numerous that the endothelial cells could no longer take care of them. Werigo found anthrax bacilli to disappear from the circulation within a few minutes. They were held by phagocytes in the spleen, liver and lungs. Adami reports similar reactions with various pathogenic bacteria. Bull, studying the fate of typhoid bacilli when injected intravenously into normal rabbits, found that the bacilli were phagocytosed by polymorphonuclears which had accumulated in the capillaries of the lungs, liver and spleen. There was no phagocytosis of bacilli in the blood. In discussing this point he states:

We have found no indications that phagocytosis of the bacteria studied by us takes place in the blood or on a grand scale in the unagglutinated state. Hence, as the bacilli cannot be agglutinated and removed by the organs and also cannot be phagocytosed in the blood stream, they continue to circulate, under some conditions, until they are removed and destroyed by the phagocytes.

Bull also studied the blood of rabbits in which bacteremia had been allowed to develop for ten hours after the injection of virulent pneumococci. The injection of immune serum was followed by immediate clumping and removal from the circulation of diplococci by the lungs, liver and spleen. In this case the organisms were also phagocytosed by polymorphonuclears which had accumulated in the capillaries, sinusoids and blood spaces of these organs. "The act of phagocytosis follows quickly upon the agglutination and removal and, it would appear, never takes place in the blood stream itself; for in the study of hundreds of blood films from the heart only one leucocyte containing diplococci was encountered."

In Rosenthal's experiments only a few of the polymorphonuclears of the capillaries and blood spaces of the organs took part in the process of ingestion, the reticular and endothelial cells being the most active phagocytes. Wyssokowitsch ('86), who was the first to make a detailed study of experimental bacteremia, found *Micrococcus tetragenous* and the typhus

bacillus to be phagocytosed exclusively by the endothelial and reticular cells of those organs in which the velocity of the blood current is greatly reduced, i.e., of the spleen, bone marrow, lungs and kidneys. He used various kinds of organisms, but never found the polymorphonuclears to be active, and never found a case of phagocytosis in the general blood stream, although particular attention was given to a study of blood films. Great variation in the time of disappearance of different organisms from the blood stream was noted, from which it was concluded that the nature of the substances given off by the organisms was of great importance in determining the phagocytic activity of the cells of the organs.

These experiments, and those of Bull, Rosenthal, Metchnikoff and others show that when living organisms are injected into the blood stream they are removed either by the cells lining the blood channels of certain organs or by leucocytes which have accumulated in those organs, but never by the leucocytes in the general circulation. The type of cell involved in phagocytosis seems to depend altogether on the organism used, and a certain amount of isolation from the blood stream is necessary before phagocytosis can take place.

Experiments to be described below show that the type of phagocytic cells involved in the process of taking up unorganized foreign matter depends altogether on the conditions of the experiment. According to Buxton and Torrey this is also true when living organisms are used. Typhoid bacilli and staphylococci injected into the peritoneal cavity of rabbits appeared within macrophages of the mediastinal lymph nodes within one hour. The authors conclude that the macrophages have seized the organisms because they were present before the arrival of the polymorphonuclears. Later the latter invaded the nodes in great numbers and seized whatever organisms were left.

The power to phagocytose may vary greatly in cells of the same type (Hektoen, Rosenow), and cells which are not ordinarily phagocytic may become so under certain conditions, as shown by Achard, Raymond and Foix, who reported a case in which the eosinophils of a pleural exudate were very active as phago-

cytes. Phagocytic eosinophil leucocytes have also been reported by Lattan-Larrier and Parvu, Wendenburg, and by Weinberg-Séguin.

From the above it is evident that phagocytosis is a physiological process which is not confined to any one type of cell, and that the material to be phagocytosed which under ordinary conditions is taken up by a certain type of cell may, under slightly different conditions, be taken up by cells which genetically and structurally are quite distinct from the first type. As examples from Pathology may be cited the well known 'Herzfehlerzellen'—epithelial cells from the alveoli of the lung which have phagocytosed red corpuscles, the neuroglia cells of the nervous system which may become very active phagocytes, and even the ganglion cells of the nervous system which may phagocytose leucocytes when inflammation of the meninges is accompanied by pus formation. Many other examples of this type of pathologic phagocytosis may be gleaned from any good text book of Pathology.

If vital staining with the colloidal dyes is merely a process of ingestion (Evans-Schulemann) then we should expect to find the same variations in the reactions to the dyes that we find with phagocytosis of other kinds of foreign material. Rosenthal and others have shown that phagocytosis of foreign material does not take place within the general circulation under ordinary conditions unless the blood is flooded with so much of this material that it cannot be eliminated within a relatively short period of time, and this in spite of the fact that the blood contains plenty of cells which are known to be active phagocytes. As is to be expected, the same results are obtained when the colloidal dyes are used as the foreign material. There is no ingestion of the dye on the part of cells already present in the blood stream, but it is taken up very quickly by the reticulo-endothelial cells of the liver, lymph nodes, spleen and bone marrow, and by clasmatoocytes, etc., after the stain has diffused into the tissues. The only difference between this reaction and that with which we are familiar when other kinds of nontoxic material are injected is the fact that the colloidal dyes which

are commonly used diffuse readily into the tissues, resulting in a more active phagocytosis of their particles on the part of tissue cells than is the case when less diffusible substances are used. Living organisms are disposed of by the reticular and endothelial cells and so do not get a chance to reach the clasmatoocytes and other phagocytic cells of the tissues.

The same material injected into the tissues may cause active migration of the phagocytic cells from the blood stream to the site of injection, where they will soon begin to ingest the foreign substance. This well known fact evidently indicates that conditions in the circulating blood stream are not favorable for phagocytosis, and it also shows that cells which will not take up foreign material from the blood stream will readily do so after they have migrated to the tissues.

Different types of phagocytic cells show a decided preference for certain kinds of foreign matter, and for this reason Metchnikoff divided them into two groups of 'microphages' and 'macrophages.' We now know that one group may act as a substitute for the other if it happens to be the first in the field, and we also know that cells which ordinarily are not phagocytic may become so under certain conditions (phagocytic eosinophils, etc.).

Exactly the same variations are seen when we use the azo dyes as the material to be phagocytosed. The results depend altogether on the conditions of the experiments. No cells of the circulating blood will be stained when the dyes are injected intravenously, but tissue cells, both fixed and free, are seen to be loaded with the dye granules. Very few of the free cells of the peritoneal cavity will contain the dye, but if the injection is made directly into the peritoneal cavity most of its free cells will contain the granules. This difference in the behavior of the peritoneal cells, depending on the method of injection, has already been pointed out by Pappenheim and by Tschaschin. It is also seen that more of the cells of the milky patches of the omentum will contain the dye following intraperitoneal injection than is the case when the dye has been injected into the veins. With subcutaneous injections of the dye we find that

most of it is contained in large cells of the macrophage or polyblast type which are presumably all of tissue origin. However, we also find plenty of smaller cells with all the morphological characters of lymphocytes which also contain the dye in the form of granules. Aschoff-Kiyono, Schulemann-Evans and others count all of these cells with the group of 'histiocytes' or 'macrophages,' thus indicating their belief that these cells are always of tissue origin. They are supposed to have no immediate genetic relationships with lymphocytes which might have migrated from the blood vessels. However, with the work of Tschaschin, and the studies of Maximow with tissue cultures of lymph nodes, together with the variations in the reported behavior of the free cells of the peritoneal cavity, depending on the method of injection, before us we are not so sure that lymphocytes are not concerned in the reaction. It is quite possible that many of the large polyblasts of the subcutaneous tissue are enlarged phagocytic lymphocytes which have migrated from the vessels.

The above experiments indicate that under the special conditions with which they were carried out the dye is preferred by the cells which Metchnikoff included in his group of macrophages, and also by all of those cells, both fixed and free, which H. M. Evans has included in the group. The experiments do not prove, however, that this group of cells is entirely separate from and genetically independent of the lymphocytes from the parenchyme of the lymphoid organs and the blood stream. On the contrary, we already have many observations which seem to indicate that the lymphocytes from the blood should be included in the group. The fact that these lymphocytes will not take up the Pyrrholblau while they are still in the circulation is of no significance, for we know that the polymorphonuclears which are active phagocytes for most kinds of bacteria and for other foreign matter will not become active until they have reached the tissues.

It is known that the polymorphonuclears may phagocytose material which under ordinary conditions is taken up by the large mononuclear macrophages, as, for example, in the experi-

ments of Schott with the injection of foreign erythrocytes into the peritoneal cavity, which resulted in the phagocytosis on the part of a few of the polymorphonuclears present of the erythrocytes and their fragments. If vital staining with the azo dyes is a matter of phagocytosis rather than of true staining of pre-formed structures it should be possible to induce the polymorphonuclears to take up the dye under similar conditions. The other phagocytic cells of the blood, the large mononuclears and larger lymphocytes, should also phagocytose the dye under the same conditions.

The loose subcutaneous tissue is not a very favorable tissue for such experiments, because it contains so many clasmotocytes and wandering cells of other types which proliferate rapidly after the injection of the dye that the cells which migrate from the blood vessels get little chance at it. Polymorphonuclears are numerous within five hours after the injection, but none of them contain dye granules, because the clasmotocytes and polyblasts present have already taken up the dye. This is probably due to the fact that the dye diffuses rapidly over a comparatively wide area, bringing many macrophages which are already present in the tissue in contact with it.

If a less diffusible substance, such as ordinary carmine ground up in salt solution, is used the results are at first very different. The salt solution filters out through the surrounding tissue, leaving the carmine granules in a dense mass at the point of injection. This mass is invaded by polymorphonuclears which rapidly phagocytose the granules. However, even in this case, those macrophages at the edge of the mass which come in contact with the carmine show a greater avidity for it than do the polymorphonuclears. The lymphoid mononuclears gradually increase in number and invade the mass of carmine. The carmine spreads, probably largely through the agency of the polymorphonuclears which drag it into the surrounding tissue. The polymorphonuclears degenerate, at the same time liberating the carmine. This is taken up by the macrophages which, in the mean time, have become very numerous. In the later stages all

of the carmine is contained in the lymphoid mononuclear cells and all of the polymorphonuclears have disappeared.

In the peritoneal cavity Pappenheim-Fukushi report the presence of carmine granules in polymorphonuclears following the intraperitoneal injection of lithium carmine. This seems to be rather exceptional for the peritoneal cavity, probably because of the normal presence of numerous cells belonging to the macrophage series.

The intramuscular connective tissue contains very few clasmatocytes and free lymphoid wandering cells, and hence when the dye is injected there it is not disposed of as rapidly as in the subcutaneous tissue. The density of the tissue prevents rapid diffusion. The result is, that when the polymorphonuclears appear on the scene there is still plenty of free dye present. The leucocytes being thus in direct contact with the dye are able to phagocytose it.

The polymorphonuclears store the dye in exactly the same form as it is stored in the macrophages or histiocytes. It is concentrated in the form of large, irregular blue granules identical in appearance with the dye granules of the macrophages of the subcutaneous tissue or of the reticulo-endothelial cells of lymph nodes. Polymorphonuclears with dye granules from the intramuscular tissue of a white rat are shown in figure 3. That the 'dye granules' are not the specific granules which are seen when a blood smear is stained with an ordinary blood stain is shown by a comparison of the dye granules of figure 3 with the granules of the polymorphonuclears from the circulating blood of the same animal shown in figure 1. Because it has become flattened during the process of making the smear the latter cell appears much larger than the cells from the section of the intramuscular tissue. Nevertheless, its granules are evidently much smaller than are the dye granules of the more compact cells from the sections of the muscle.

The muscle sections contain a few cells having the general characters of medium-sized and larger lymphocytes, excepting that they contain typical dye granules. According to Aschoff-Kiyono they are therefore 'histiocytes' of tissue origin, and not

lymphocytes. Their number is so small that it is impossible to determine from this experiment whether or not they have migrated from the vessels. The next experiment, however, shows that they might be true lymphocytes from the blood stream.

This experiment consisted of the injection of Pyrrholblau into the doubly ligatured femoral vein of a white rat. The ligatures were placed first, and the dye was then injected into the lumen of the vessel between the two ligatures. The next day the isolated segment was removed, fixed in Helly's Zenker-formol mixture, imbedded and sectioned. Figure 4 is a drawing of a low power view of a portion of one of the sections. All of the polymorphonuclears which came in contact with the dye are loaded with it, and it is concentrated in the form of the typical 'dye granules.' There is no diffuse staining of cytoplasm or nucleus, which indicates that the leucocytes were living at the time of fixation. The nuclear stain was obtained by counterstaining the sections with hemalum. Without a counterstain the nuclei are absolutely colorless, while the blue granules are very conspicuous. The granules are identical with those of the polymorphonuclears of the sections of muscle.

The low power drawing (fig. 4) gives a good idea of the large number of polymorphonuclears present in the vessel. It is quite evident that many of them have migrated in from the surrounding tissue or from the vasa vasorum. There has apparently been no increase in the number of lymphocytes, for they are not more numerous than would be expected in the amount of blood included in a section through the vein.

The large and medium-sized lymphocytes in the vessel also contain the dye granules, as is seen in figure 2, and a few small lymphocytes were found which contained one or two dye granules. This seems to dispose definitely of the idea that lymphocytes cannot take up the dye granules, for in this case we are not dealing with 'histiocytes' from the tissues.

This and the previous experiment have shown clearly that the polymorphonuclears, cells which are undoubtedly blood cells, may take up the colloidal dyes when they are isolated from the general circulation, and the experiment with the ligatured vessel

proves that lymphocytes which are already present in the blood will also take up the dye when a condition of stasis is brought about by the application of the ligatures.

These results are exactly what might be expected when they are considered in the light of what is known about phagocytosis in general. For example, in bacteremia it seems that phagocytosis of the bacteria in the general circulation is of rare occurrence, but in the capillaries of the lung, and liver, and blood spaces of the spleen and bone marrow the process may be very active. Here the organisms are disposed of by the leucocytes or by the endothelial and reticular cells. In these situations the blood current is slowed down, and the conditions of the ligatured vessel are to a certain extent duplicated.

Experimental pneumonia in animals stained *intra vitam* with Trypanblau, as reported by Kline and Winternitz, is of interest in this connection. Polymorphonuclears containing dye granules were found in the alveoli, bronchioles and blood vessels of the involved lung but not in the general circulation. Injection of the blood vessels of the involved lung showed that they were cut off from the general circulation by plugs of fibrin in the capillaries.

These facts show clearly that blood cells, polymorphonuclear leucocytes as well as lymphocytes, will take up the colloidal dyes and store them in the form of coarse 'dye granules' whenever the cells become isolated from the general circulation. The cells may leave the blood vessels and phagocytose the dye in the tissues, or they may take it up while still within the vessels in case the latter have become isolated from the general circulation. The reaction to the colloidal dyes, therefore, does not differ from that which results from the injection of other foreign material including nonvirulent living organisms. Slight differences in detail due to the diffusibility of the substances injected, etc., are of no significance.

It is evident that the reaction to the colloidal dyes is no more specific than is general phagocytosis. It is true that a given type of phagocytic cell may show preference for a certain kind of foreign material, but under slightly different conditions the

same material may be taken up by cells of a different type. It has been shown that the same is true when the azo dye Pyrrholblau is used as the foreign material. The reactions of cells to this dye are, therefore, not sufficiently specific for determination of genetic relationships.

The fact that lymphocytes will take up the dye from the ligatured vessel shows that many of the wandering cells of the connective tissue may be lymphocytes which have come from the vessels. The presence of dye granules in a wandering cell of the connective tissue is no proof for its histogenous origin. It may be one of the larger lymphocytes which has migrated from the vessels and taken up the dye without further differentiation, or it may be a lymphocyte which has differentiated into a 'polyblast' (Maximow, Tschaschin).

The lymphocytes containing dye granules are usually the larger lymphocytes and large mononuclears which do not seem to have undergone any further differentiation into 'polyblasts.' This is contrary to the findings of Maximow in tissue cultures from lymph nodes, and it seems to indicate that the lymphocytes of the blood are more mature than are those of the nodes. Tschaschin concluded that they must leave the vessels before they are capable of taking up the colloidal dye. This evidently is not the case, but isolation from the general blood stream is necessary. The special conditions of the tissues are duplicated in isolated vessels, at least in so far as phagocytosis is concerned.

In the lymphoid organs the lymphocytes seem to be too immature to be able to act as phagocytes. In the tissue cultures (Maximow) they are capable of further differentiation and of phagocytosis, but it is doubtful whether this is possible in the lymph nodes under ordinary conditions. According to Kiyono they may undergo further differentiation in the later stages of aseptic inflammation and it is then impossible to distinguish the larger ones from histiocytes (Kiyono). Babkina states that they may differentiate into 'polyblasts' under these conditions, and Maximow has found these polyblasts to take up colloidal dyes in tissue cultures.

The chemical and mechanical conditions of the blood stream are not favorable for phagocytosis which has been noted here only under exceptional conditions. The same conditions which prevent mitosis of lymphocytes in the circulating normal blood may also inhibit phagocytosis and prevent further differentiation of these cells as long as they are in the blood stream. In the thoracic duct mitotic figures are numerous and many of the cells contain ingested erythrocytes, etc., but when they reach the blood stream their phagocytic and proliferative activity is temporarily suspended. The constant churning action to which the cells are subjected in the general circulation may also have the effect of slowing up any phagocytic activities on their part. Under these conditions the cells are probably not in contact with the foreign particles for sufficient length of time to be able to phagocytose them. This would be especially true of substances like the azo dyes, which are eliminated from the circulation very quickly. There is more chance for phagocytosis if the substance remains in the circulation for a long period of time, as is the case when carmine is injected. Hoffmann and Langerhans report the presence of carmine as late as 148 days after injection. They could find none of it in the lymph nodes until three days after the injection, which indicates that this substance is eliminated from the circulation very slowly. In the blood it is taken up by the "ordinary white corpuscles."

Although it is difficult to determine the exact conditions which influence phagocytosis in the blood stream, it seems likely that the time during which the foreign substance remains in the circulation, as well as chemical and physical conditions, is of importance. Isolation of a segment of a vessel from the general circulation, or migration of the leucocytes and lymphocytes from the vessels places these cells under such favorable conditions that they are soon able to begin their special activity as phagocytes.

Both lymphocytes and polymorphonuclears take up Pyrrholblau from a ligatured vessel, and the intramuscular tissue also contains cells of both types with dye granules. We know that polymorphonuclears of this location have come from the vessels,

but it is difficult to prove that any of the lymphoid cells with dye granules have also migrated from the vessels. However, since the lymphocytes of the ligatured vessel take up the dye very freely it is more than likely that many of those which migrate to the tissues will also phagocytose the dye. The larger ones can probably take it up immediately, but the smaller ones would naturally require some time for further growth and differentiation. This would explain the conditions in the *tâches laiteuses* of the omentum in vitally stained animals. The smaller and more immature lymphocytes in the central portion of the nodules take none of the dye, while the larger and more highly differentiated ones in the peripheral regions take it up very freely. Since it has been shown that the lymphocytes of the blood may take up the azo dye there is no longer any reason for separating the peripheral cells as 'histiocytes' from the true lymphocytes. They are all lymphocytes, and in so far as their reaction to the dye is concerned it makes little difference whether they are of tissue origin or whether they have recently migrated from the vessels. Their state of differentiation and the special conditions under which they happen to exist at the time are of chief importance in determining their reaction to the dye.

Full consideration of the results of these experiments adds new and strong evidence in favor of the view of Schulemann-Evans, that vital 'staining' with these dyes is primarily a process of ingestion or phagocytosis, at least in so far as the cells considered here are concerned. If we were dealing with a process of true staining of preformed structures of the cell it would be difficult to account for the variations in the reaction under different experimental conditions. Whether the ingested colloidal material finally combines with constituents of the cell protoplasm is another question and one which can not be settled with experiments of this nature. The impression gained from working with a considerable mass of material is, that the dye granule is more than a mere concentration of dye particles in a cytoplasmic vacuole. The granules become very firm and resistant to reagents; they may be stained, and in other respects their behavior is more like that to be expected of a structural

constituent of the cytoplasm. Phagocytosis, however, seems to be the process which gets the dye into the cytoplasm, and the dye granule which results is certainly not a preformed structure, for such granules cannot be demonstrated with any other methods.

Admission that the process is one of ingestion and storage rather than of true staining forces the giving up of the idea of the specific nature of the reaction, for we have seen that phagocytosis is not a specific reaction confined to any one group of cells. We are, therefore, no nearer to the solution of the problems for which the method has been used than we were before the advent of the azo dyes. The attempt to classify cells according to their reactions to the colloidal dyes must, therefore, be regarded as a failure.

SUMMARY

1. Intra vitam 'staining' with the azo dye Pyrrholblau is a process of ingestion and storage, and not one of true staining of preformed structures.

2. Blood cells, both lymphocytes and polymorphonuclears, will ingest and store the dye after they have migrated into the tissues, or when the blood vessel which contains them has been isolated from the general circulation. In this respect the blood cells behave just as they do toward living organisms or other foreign material.

3. The method is not specific and cannot be used for the purpose of distinguishing between cells of tissue origin and those which have come from the blood stream.

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PLATE

PLATE 1

EXPLANATION OF FIGURES

1 Polymorphonuclear leucocyte from blood smear of albino rat. The granules are the specific granules of these leucocytes, and not dye granules.

2 Dye granules in lymphocytes from section of doubly ligated femoral vein of albino rat.

3 Dye granules in polymorphonuclears from section of rat muscle injected intra vitam with Pyrrholblau.

4 Portion of a section of the contents of doubly ligated femoral vein of rat. Intra vitam injection of Pyrrholblau between the ligatures. Vein removed and fixed twenty hours later. Dye granules in the polymorphonuclears. Dye granules in lymphocytes of this same vessel are shown in figure 2.

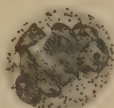


Fig. 1



Fig. 2



Fig. 3

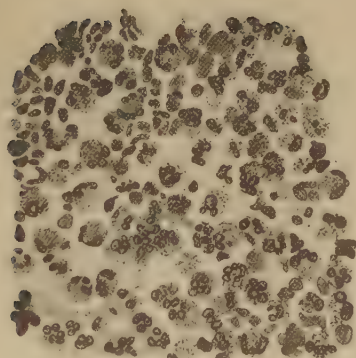


Fig. 4

Helen A. Sanborn, del.

OBSERVATIONS ON THE OCCURRENCE OF EOSINOPHILIC LEUCOCYTES AND THE GRANULE CELLS OF PANETH IN THE VERMIFORM APPENDIX OF MAN

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The comparative infrequency of careful descriptions of the occurrence and distribution of many cell types encountered in the vermiform appendix of man, has led us to make a study of this organ. This is all the more important, since an accurate knowledge of the normal cellular content of this organ is essential in making a study of many of the less definite pathological reactions which occur in the appendix. This paper embodies the results of a careful analysis of specimens of normal appendices with regard to the presence of Paneth's cells and the normal content of eosinophilic leucocytes. These specimens have been obtained as a result of the routine removal of this organ in instances of laparotomy for abdominal and pelvic lesions other than those of the appendix. Only those specimens have been selected that are free from angulations, bands of adhesion, dilations, obliterations, lymphoid destruction, parasitic worms, and inflammatory exudates.

EOSINOPHILIC LEUCOCYTES

Although it is generally recognized that eosinophiles are normally found in the mucous membrane of the alimentary tract, there are only a few references to the number, distribution, and frequency of the occurrence of these cells in the vermiform appendix. According to Schwarz ('14), Oehler ('12) found eosinophiles very frequently in the vermiform appendix of man; Loele ('11) and Aschoff ('08) also have mentioned the great numbers

found in this organ. Aschoff, moreover, has noted that while the appendix of the newborn contains only a few of these cells, in the suckling there is an increasing number and in the appendix of the adult a great many eosinophiles. For a careful and extensive review of the literature dealing with cells containing eosinophilic granules, the reader is referred to the monograph by Schwarz.

It is not the object of this paper to go into the nature of the eosinophilic granules, or the significance of the presence of these cells in the appendix, but rather to describe the frequency of their occurrence, their distribution and their origin. These observations are based upon the study of sixty normal appendices taken in the consecutive order as they came into the laboratory of pathology.

These specimens were fixed in 10 per cent commercial formalin, 10 per cent formalin neutralized over magnesium carbonate, Zenker's solution and Bensley's fluid. Paraffine sections of 5 microns were prepared. These were stained with hemotoxylin and eosin and with the molybdenum alizarin lac method of Okajima ('16). The Winkler reaction was used as follows for the demonstration of oxidase granules in the eosinophilic leucocytes. After formalin fixation frozen sections and paraffine sections brought down into distilled water, were treated for two minutes with the following freshly prepared mixture: 1 per cent alpha-naphthol in a 1 per cent KOH added to an equal quantity of a 1 per cent aqueous solution of dimethylparaphenyldiamin hydrochloride. They were then washed and mounted in distilled water. All oxidase granules appear a blue-black. Under a 4 mm. objective, camera lucida drawings of the cells in areas reacting positively to the Winkler test were made. Cover glasses were lifted off the sections and the indophenol blue was dissolved by the means of alcohol. Sections were then returned to water, stained with hemotoxylin and eosin and mounted in balsam. By superimposing upon the former drawing, it was possible to identify the cells containing the oxidase granules. These with very few exceptions were eosinophilic leucocytes. It is interesting to note in this connection that in

the study of these specimens and many other tissues, both normal and pathological, we have found that in paraffine sections the eosinophile is the only cell in which granules of indophenol blue can be satisfactorily demonstrated.

The eosinophiles are found in relatively large numbers in the tunica propria and submucosa. With the Winkler reaction, the boundaries of the glands and the lymph nodules are distinctly marked by one or more rows of indophenol blue containing cells which prove to be eosinophiles. Frequently these cells are seen migrating through the epithelium. They have never been observed in this series within the lymphoid nodules. Rarely are they found in the muscular coats, but occasionally are encountered in the serous coat. The vast majority are found free in the tissues but they are sometimes seen within the blood vessels. Eosinophiles were found in every one of these sixty specimens which had been selected on account of their normal character. The majority of these cells present typical indented or polymorphous nuclei although some of them contain spherical nuclei.

While experimenting with an alizarin stain as described by Okajima ('16) it was applied to sections of several of these appendices. This stain is supposed to be elective for hemoglobin. It was found that the granules of the eosinophiles took the stain equally as well as the erythrocytes. This brings up the question again as to whether the eosinophilic granules are of hemoglobinous nature. Either this is the case or the stain is not an elective stain for hemoglobin.

To determine whether the eosinophiles found in the stroma of the appendix are formed in situ or are of myeloid origin, the indophenol blue synthesis, or Winkler reaction, was used as outlined above. It appears definitely established by the work of a number of German investigators that indophenol oxidase granules are found after formalin fixation only in the lachrymal and thyroid epithelium and in cells of myeloid origin. It has been used in this country by Evans ('15, '16 a, b, c, d) upon blood smears and exudations in a study of the mononuclear leucocytes and also in a study of the reaction found in acute

inflammatory tumors of spleen. The reader is referred to the above papers by Evans for a review of the literature. Recently Forman and Warren ('17) have used it as a means of identifying the tumors of myeloid origin. After applying the Winkler reaction upon sections of these appendices, it was found that with the exception of an occasional neutrophilic polymorphonuclear or mononuclear leucocyte every cell containing the indo-phenol blue was an eosinophilic leucocyte. It was also found that the indophenol blue must be dissolved with alcohol before the α -granules would take an eosin stain. It would appear, therefore, that the indophenol blue was formed in such a way as to protect the α -granules from subsequent staining. If the Winkler reaction is applied after the eosin, however, it is found that the eosinophilic granules are obscured by the formation of the indo-phenol blue. The eosinophilic leucocytes free in the tissues react in the same manner as do those that are found in the lumina of the blood vessels. The above findings warrant the conclusion that the eosinophilic cells found normally in the appendix are of myeloid origin.

THE GRANULAR CELLS OF PANETH

Paneth's cells have been described as occurring in a great many of the mammals as well as in some of the lower vertebrates. It is generally recognized that these cells occur at the base of the glands in all portions of the small intestine in man. They, however, have been described by some as occurring in the glands of the stomach. Bloch ('03) has described them in glands of the large intestines of nursing infants. Schmidt ('15) on the other hand, failed to confirm Bloch's observation but did describe them as occurring in the vermiform appendix. He examined 66 specimens and found them in 30 instances. Age appeared to have no influence upon their number. They did vary greatly as to the number present in the several specimens. This is the only reference to the occurrence of the cells of Paneth in the appendix of man with which we are familiar. Klein ('06) observed that, in the opossum, these cells occurred not only in the basal portions of the glands of the small intestine

but also on the sides of the villi. He, further, demonstrated that, in the guinea pig, the granule content of the cells bore a relation to the taking of food.

During the progress of the study of the presence of eosinophiles in the vermiform appendix, the routine fixation methods were changed from 10 per cent commercial formalin and Zenker's solution to 10 per cent formalin neutralized over magnesium carbonate and Bensley's solution made by adding 10 cc. of neutralized formalin instead of glacial acetic acid in the Zenker formula. It was in this way that our attention was called to the presence of Paneth's cells in this organ. A neutral fixative appears essential for a sharp staining of the granules of these cells.

In sections stained in hematoxylin and eosin after a neutral fixative, the granules in the Paneth cells stain a brilliant red. It was found advisable not to draw the hematoxylin with acid as it renders the granules less distinct. A better method of staining, however was the use of iron alum hematoxylin followed by mucicarmine for ten minutes. The granules stained black while the mucin of the goblet cells stained red or pink.

The granular cells of Paneth were found in each of the eleven normal appendices examined after the neutral fixation. The number varied in the different specimens. In some instances only one or two cells were found after examining several sections. Examination of other specimens revealed many of these cells in a single section. Their distribution appeared to be uniform throughout the appendix. Sections made from the distal, middle and proximal portions presented approximately the same number of these granular cells. These cells were uniformly situated in the base of the glands.

The presence and number of these cells in the specimens examined did not appear to bear any relation to age. These findings agree with those of Schmidt except that these cells occur more constantly in our somewhat smaller series. On account of the nature of the material, no attempt has been made to determine whether the number of granules bore any relation to the ingestion of food. It, however, is to be remembered that these specimens were from surgical patients who were fasting.

It is not the purpose of this paper to enter into a discussion of the significance of the presence of the cells which have been shown by Klein to be concerned with secretion in the intestine of the guinea pig and to be increased in this animal during fasting periods.

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AN ANATOMICAL CONSIDERATION OF THE CEREBRO-SPINAL FLUID

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INTRODUCTION

Although an extensive literature dealing with the cerebro-spinal fluid and its meningeal pathways has developed during the last forty years, there still seems evident a lack of a comprehensive understanding of the anatomical relations of these fluid-channels. While it may be unfair to judge the trend of anatomical knowledge and progress by the viewpoints expressed in the text-books in this subject, still the many inaccuracies and disagreements concerning the processes of the cerebro-spinal fluid appearing in the more recent of these publications seem hardly warranted. The chief difficulty in all the writings deals with the relationship and possible connections of the meningeal channels with the true lymphatic system. The whole consideration of the cerebro-spinal spaces as presented in current treatises suggests strongly the anatomy of true coelomic serous cavities. Quite apart from the perhaps pardonable ignorance regarding the anatomical aspects of the circulation and distribution of the cerebro-spinal fluid, is an even greater confusion about many of the problems of these fluid-retaining membranes, due in part at least to a misunderstanding of the physiological requirements of such pathways.

A common cause of error in the discussions of the cerebro-spinal spaces has been the abundant references to the fluid of these spaces as 'lymph.' The earlier usage of the word 'lymph' as meaning almost any body-fluid has largely been done away with in physiological writings and today a distinction should

surely be made between tissue-fluid¹ (extravascular lymph?) and the liquid of lymphatic vessels (lymph). This change in the viewpoint in regard to the more restricted use of the word 'lymph' has been largely brought about by the demonstration that the lymphatic vessels are closed tubes and are not connected directly by any openings to tissue-spaces or serous cavities. While physiologically, an apparently direct communication may be afforded for the passage of fluids into the lymphatics, anatomically the lymphatic system is wholly closed. Not only anatomists but physiologists and pathologists have offended by using the word 'lymph' to refer loosely to the cerebro-spinal fluid as well as to most of the other body-fluids. It may be said that the fluids of the body have thus been grouped together, largely because of ignorance regarding their exact functions and processes.

The cerebro-spinal fluid in its physical and chemical characters, is a fluid wholly different from the lymph of the true lymphatic vessels. Halliburton ('04) described this fluid as being a clear limpid liquid of a low specific gravity (1.004 to 1.006), containing few cells and little protein, with a small salt content and possessing dextrose in definite traces. Very recently, this same investigator ('16), in his presidential address before the Royal Society of Medicine, likened the cerebro-spinal fluid to an ideal physiological salt solution. At the same time, Halliburton advanced arguments for the physiological conception that this fluid subserved a definite function in the nourishing of the nervous tissues, and that only in such a restricted sense could the fluid be called the lymph of the brain. But a close and careful differentiation between the chemistry of this fluid and of the lymph of systemic lymphatic vessels was strongly emphasized by this worker. Mott ('10) in his Oliver Sharpey lectures, and

¹ This term was used by Sabin in her Harvey Lecture on "The method of growth of the lymphatic system" (Harvey Lectures, Series IX, New York, 1915-1916) to designate the fluid of the tissues. The term 'tissue-juice' was not employed as it has so frequently been applied to the fluids obtained by subjecting tissues to great pressures in a Buchner press. The term 'tissue-fluid' seems very appropriate.

Cushing in a more recent paper ('14) have likewise presented excellent conceptions of the fluid from somewhat different aspects.

The importance of the cerebro-spinal fluid in the clinic has been recognized since Quinke demonstrated the ease of obtaining it by lumbar puncture. Pathologically the meninges and the subarachnoid fluid have received a great deal of attention, and since the time of Key and Retzius ('76), many observations have been made upon the physiology of the fluid. But the anatomy of the cerebro-spinal spaces (i.e., the pathways for the cerebro-spinal fluid) has not until recently received its due measure of attention. This avoidance of an anatomical viewpoint in the investigations in this field seems to offer a justification for this article. For it is proposed here to present merely a brief account of the anatomical relationships of the pathways for the cerebro-spinal fluid: to present the cerebro-spaces from the aspect of the functional employment of these channels. While logically this presentation should deal first with the embryology of these fluid-pathways about the nervous system, it seems best to bring up to the present the conception of the adult anatomy of this region and then proceed to the developmental problems of the subject.

THE INTRAVENTRICULAR SOURCE OF THE FLUID AND ITS INTRA-VENTRICULAR COURSE

The first hypothesis regarding the origin of the cerebro-spinal fluid may be referred to Haller (1757) who considered it to be the product of the leptomeninges. This belief in regard to the source of the fluid was also expressed by Magendie ('25). Faivre in 1853 and Luschka in 1855 independently suggested the choroid plexuses of the cerebral ventricles as the elaborators of the fluid. The evidence for this view was for a time solely histological and the hypothesis was not placed on any firmer basis until nearly fifty years afterward. The fact that these villous projections into the ventricles possessed a glandular morphology, however, caused a wide acceptance of this conception. Pathologically, also, the intraventricular origin of the fluid seemed established in those cases of hydrocephalus subsequent

upon obstruction within the aqueduct or other interventricular channel.

More convincing proof of the production of cerebro-spinal fluid by the chorioid plexuses was given by the observations of Cappelletti ('00 a, b) and of Pettit and Girard ('02). The first of these workers was able to increase the rate of flow of fluid from a cannula after the administration of pilocarpine, muscarin and ether. Pettit and Girard described for the first time histological changes in the cells of the plexus indicating an augmented production of fluid. Following these important observations, a number of other observers, particularly Meek ('07), Mott ('10), Pellizzi ('11 a, b) and Hworostuchin ('11) have added histological data in support of this viewpoint. Findley ('99), shortly before the action of drugs was tried, presented an excellent pathological consideration of the plexuses. Dixon and Halliburton ('13) have demonstrated changes in the rate of production of the fluid after injection of certain tissue-extracts and of drugs.

Somewhat more definite proof of the relation of the chorioid plexuses to the elaboration of the cerebro-spinal fluid has been offered by Dandy and Blackfan ('13, '14) who produced hydrocephalus by blocking the aqueduct. Removal of the chorioid plexuses in such an experiment prevented the development of the hydrocephalus. In another way, the writer ('14c) was able to show varying rates of elaboration of the fluid when a catheter was inserted into the third ventricle through the aqueduct, demonstrating that the increased elaboration of fluid was not to be referred to an increased permeability of the cerebral capillaries, and also that the intraventricular production of fluid could be influenced by mechanical (vasomotor) and chemical means (Weed and Cushing ('15)).

From this evidence—histological, pharmacological and physiological—the function of the cells of the chorioid plexuses as the elaborators of a characteristic body-fluid, the cerebro-spinal fluid, seems established. It must be understood, however, that these structures, while undoubtedly producing by far the greatest portion of the cerebro-spinal fluid, constitute merely the in-

traventricular mechanism for fluid-elaboration. There is, also, further production of cerebro-spinal fluid by the nervous tissue itself—a small addendum poured by way of the perivascular channels into the subarachnoid spaces. This mode of fluid-manufacture will be discussed in a later paragraph. Furthermore, a minimal production by the ependymal cells, negligible in its significance and total amount, may occur.

The cerebro-spinal fluid, elaborated by the chorioid plexuses, is poured into the cerebral ventricles, which are lined by smooth ependyma. That portion of the fluid formed in the lateral ventricles escapes into the third ventricle and thence by the aqueduct into the fourth ventricle. Likewise, an ascending current of fluid apparently occurs in the central canal of the spinal cord; this, representing a possible product of the ependyma, may be added to the intraventricular supply. From the fourth ventricle the fluid is poured out into the subarachnoid spaces; there is no evidence that functional communications between the cerebral ventricles and the subarachnoid spaces exist in any region except from the rhombic ventricle.

Some uncertainty as to the exact mode of escape of the cerebro-spinal fluid from the fourth ventricle into the subarachnoid spaces still persists. The weight of evidence surely inclines toward the consideration of the foramen of Magendie as a true functioning communication in the inferior velum. This medial foramen has been termed by many observers an artifact, but developmental observations—those of Hess ('85) particularly and of Blake ('00)—indicate that there is a true anatomical break in the membrane. The findings of Cannieu ('97) and of Wilder ('86, '93) should be included here in support of the actual existence of this medial opening. Blake's conception of the formation—a shearing off at the base of a finger-like evagination of the rhombic roof—receives some support from Wilson's ('06) studies of the calamus region and the nucleus postremus. Likewise Retzius' ('96) description of a constant pial fold constituting the posterior wall of the fenestration, in addition to the variable true obex, adds support to the conception of the actual occurrence of a median foramen. The two lateral foramina,

those of Luschka, connecting the lateral recesses of the fourth ventricle with the subarachnoid spaces, seem to have as actual an existence as the medial opening. It is probably through these three foramina—or surely in the region of the inferior tela chorioidea if through an intact permeable membrane—that the cerebro-spinal fluid, produced in the cerebral ventricles, passes into the subarachnoid spaces.

THE MENINGEAL SPACES

The cerebro-spinal fluid, passing through the foramina (or through permeable membranes) of the rhombic roof, enters the subarachnoid spaces in the medial cerebello-bulbar angle. The immediate reservoir into which the fluid is poured is the cisterna cerebello-medullaris; from this region the further distribution of the cerebro-spinal fluid is accomplished. This new product of the chorioid plexus slowly diffuses downward into the spinal subarachnoid spaces, but passes more rapidly upward about the base of the brain and thence, more slowly, over the hemispheres, covering the whole cerebro-spinal axis in a perimedullary arrangement. The evidence for this extraventricular course of the fluid, as outlined, is based on the rather rapid spread of dye-stuffs throughout the subarachnoid spaces, after their introduction into the cerebral ventricles. Replacements of the embryonic intraventricular fluids likewise give indisputable support for this complete perimedullary arrangement.

In the vertebral canal and in the cranium, the anatomical characters of the subarachnoid spaces are similar. The whole space is usually described as being bounded on the outer side by the inner surface of the arachnoidea and on the inner side by the pia mater. By analogy, then, with the coelomic serous cavities, the arachnoid becomes the parietal layer of the space and the pia the visceral layer. In addition, the space is described as being interrupted somewhat by processes of the arachnoidea which run into the pia. The presence of the ligamenta denticulata in the spinal region does not in any way affect the essential anatomic relations of the space.

But such a method of description hardly takes full cognizance of the essential character of the arachnoidea and of the pia mater as constituting merely limiting structures for an intra-membranous series of channels—the subarachnoid spaces. These connected but interrupted pathways are lined by a mesothelial cell-layer—the cells being of a low, somewhat cuboidal type. These cells are probably best described as being of this low cuboidal type, following previous usage, but in places they are seemingly flattened to a pavement-type. The general morphology of the cells depends apparently not only on their situation but also on their physiological state. The outer walls of these subarachnoid spaces are constituted by the inner surfaces of the arachnoid membrane, while the interrupted side-walls of the channels are formed by the arachnoidal trabeculae. And quite similarly, the inner surfaces, or floors, of these spaces are lined by the outer cell-layer of the pia mater. Thus the subarachnoid spaces may be described as being intraleptomeningeal channels, covered on the outer surfaces by the low cuboidal mesothelium of the inner surface of the arachnoid; these cells are continued inwardly along the arachnoid trabeculae to fuse directly with the cellular covering of the pia mater, which thus forms the inner boundary. The subarachnoid space (or better, spaces) furnishes, in this way, specially organized cell-lined channels for the cerebro-spinal fluid. In certain locations, particularly in the cerebello-bulbar and cerebello-pontine angles, and in the interpeduncular space, the subarachnoid pathways become enormously dilated, with a decrease in the number of trabeculae but with no alteration of the essential morphologic relations. The largest of these spaces have been called cisternae; the smaller channels have been referred to as lacunae, flumina, etc., following the terminology of Key and Retzius ('76).

The pia mater is usually described, following the French school, as one of the three meninges closely investing the central nervous system. Its relation to the subarachnoid spaces has already been described; it forms, in reality, merely a reflection of the mesothelial cells from the arachnoid trabeculae upon the nervous tissue with a minimum of supporting elements. This membrane

should not be considered as an intact cellular layer over the neuraxis, for in places it is seemingly incomplete. Thus, in the inferior velum, the pia must be perforated if an actual medial foramen of Magendie exists, and likewise, a similar condition must hold in the rhombic lateral recesses, if the two foramina of Luschka are anatomical openings. If these areas of fluid-passage are merely permeable membranes of differentiated ependyma, morphologically intact and complete, then surely the pia over the areas must likewise assume the character of a somewhat similar, permeable membrane. Again, in relation to the perivascular channels, the pia seems perforated, in a special manner, by all of the blood vessels as they enter or leave the nervous system. In these perivascular spaces, the pia apparently enters as a mesothelial lining of the outer surface of the space; a variable distance from the exterior these cells become unrecognizable and apparently are lacking, replaced by neuroglia elements. The inner walls of these perivascular spaces seem likewise covered, for a certain distance, by mesothelial cells, reflected with the vessels from the arachnoid covering of all of these vascular channels as they traverse the subarachnoid spaces. Thus, while in general the customary description of the pia mater as a membrane closely investing the cerebro-spinal axis and plunging into the cerebral sulci holds, the intact character of the pia as a cellular layer is probably incorrect. The supporting elements of the pia will not be discussed here as they have no relation to the cerebro-spinal channels.

From some aspects, the emphasis that is laid by current descriptions upon the arachnoid as a smooth membrane bridging the cerebral sulci is quite correct. Such a character, however, belongs only to the outer surface of the meninx; there the arachnoid is a complete and intact membrane, covered with low, somewhat cuboidal mesothelial cells similar to those lining the subarachnoid spaces. Between the outer and inner layers of the arachnoidea, there is a scanty supporting tissue of white fibrils and some elastic fibers. This supporting tissue likewise forms a core for the arachnoidal prolongations to the pia mater. In view of the two different characters of the arachnoidea, it

seems best to continue to use the term 'arachnoidal membrane' to refer to the outer continuous membrane and the term 'arachnoidal trabeculae' to refer to the arachnoidal prolongations from the arachnoid to the pia mater (Weed, '16 b).

Except in certain definite areas, the arachnoidal membrane on its outer surface is entirely separated from the inner surface of the dura mater. The areas of fusion represent everywhere invasions of the pachymeninx by the arachnoidea. The most frequently found of these points of fusion are the arachnoid villi,—prolongations of the arachnoid membrane into the dura so that the arachnoidal mesothelial cells come to be directly beneath the vascular endothelium of the great dural venous sinuses. These arachnoid villi have been described in adult man, in infants, and in the ordinary laboratory mammals. Histologically these prolongations are covered by the typical arachnoidal cells (low cuboidal mesothelium) usually of only a single layer but often forming whorls of many cells and presenting double-layered coverings. The core of these villi may be a strand-like network reduplicating the arachnoid spaces (as in dog) or a myxomatous ground-work simulating the perimedullary mesenchyme. In addition to the true arachnoid villi, which occur in the walls of practically all of the dural sinuses, there are found infrequently prolongations of the arachnoid into the dura in other situations. From these other prolongations and especially from the villi, columns of arachnoid cells project varying distances into the dura (Weed, ('14 b)). The arachnoid villi represent normal structures; the great enlargement of these in late adult life in human beings results in the formation of the well-known Pacchionian granulations.

The subdural space (between arachnoid and dura) is usually considered to be a part of the cerebro-spinal channels. It is a very small space, the two limiting surfaces being separated by merely a capillary layer of fluid. Whether this fluid is exactly similar to the cerebro-spinal fluid is very difficult to ascertain. Likewise our knowledge of the connections between the subdural and subarachnoid spaces is hardly definite. Hill ('96) believed the two spaces to be intimately connected, with easy

fluid-passage through foramina or by filtration. Quinke ('72), many years before Hill, found an apparent passage of granular material only in the direction from subdural to subarachnoid. It seems established, however, that certain foreign salts, in molecular solution, introduced into the spinal subarachnoid spaces do not reach the subdural space (Weed, '14 a, b). In some ways, however, the subdural space may be likened to a serous cavity.

This analogy between the subdural space and the coelomic serous cavities is supported by the definite occurrence of flattened polygonal mesothelial cells on the inner surface of the dura. The inner limiting membrane of the subdural space is not of this character as it is covered by the somewhat cuboidal mesothelium of the arachnoid. The fluid of the subdural space, in consequence, has probably a local origin from the cells lining it, for it seems anatomically well separated from the true cerebro-spinal fluid. No proof for this view has been definitely advanced. The remainder of the dura is a thick fibrous membrane, composed almost entirely of white fibrous tissue with but a negligible amount of yellow elastic tissue. It is described as having two layers in the cranium; the evidence for this rests solely upon the fact that the fibrous membrane divides to enclose the sinuses and other structures. Anatomically in the remainder of the pachymeninx there is no line of separation. In the cranium, also, the dura subserves the function of the internal periosteum of the skull, whereas in the spinal canal, it is surrounded by the fatty areolar tissue of the epidural space.

There remains to consider, under this heading, only the permeability of these membranes, in relation to the cerebro-spinal fluid. The importance of the subarachnoid spaces as fluid-channels has already been emphasized: are these spaces efficient fluid-retainers? Evidence on this phase of the problem is afforded by the spread of injection-materials of various kinds. After injections of India ink into the spinal subarachnoid spaces, the granules of carbon are entirely retained within the same spaces if the experiment be acute; after a few hours, some of the carbon may be found in the low cuboidal cells of the arachnoid. Quite similar are the results after the injection of cinnabar; in

this case also a cellular phagocytosis of the granules takes place after some hours. These suspensions of granules are, of course, abnormal in every way; the reliable test of permeability may be made only with true solutions. If an isotonic solution of potassium ferrocyanide and iron-ammonium citrate be injected into the lumbar subarachnoid spaces, the mesothelial cells enclosing the fluid-channels, are subsequently found impermeable to the foreign salts. This may be determined by precipitating the salts as prussian-blue in an acid medium and studying histologically. In such an observation, the exposed border of the cell may be covered with the precipitate but no intracellular accumulations can be found (Weed, '14 a). Hence it seems most likely that the low cuboidal mesothelium of the arachnoid meshes forms an efficient fluid-barrier; it seems impermeable to certain foreign salts and granular material except in those cases where a specific affinity may exist. This feature of impermeability of the arachnoid mesothelium does not hold in all probability for all foreign chemicals in solution, as many of these substances are undoubtedly attracted to certain cellular elements. It is impossible at present to comment on the permeability of these arachnoid cells to colloidal suspensions. But, in general, the arachnoid channels are equipped as fluid-retainers with unquestionable powers of diffusion or adsorption in regard to certain elements in the normal cerebro-spinal fluid, deriving in this way a cellular nutrition.

THE EXTRAVENTRICULAR SOURCE

In addition to the elaboration of the cerebro-spinal fluid by the chorioid plexuses, there seems fairly well established a second source of the fluid from the nervous tissue itself. The evidence for such a view of a dual source of this fluid is rather complex and dates back to His ('65 a). The earlier experiments on the production of the cerebro-spinal fluid under the influence of drugs and other chemical substances were done without taking account of a possible increase of the flow of the fluid due to an increased permeability of the cerebral capillaries; the findings from cannulae introduced into the subarach-

noid spaces are in this respect inconclusive. There is, however, considerable evidence of value for this second extraventricular source of the cerebro-spinal fluid; some of the data supporting this conception will be presented here.

As given by Mestrezat ('12) and by Mott ('10) the fluid-spaces around the blood-vessels of the central nervous system were first described by Robin in 1858. His ('65 a) by puncture-injection was able to demonstrate a complete pericellular and perivascular network in both brain and spinal cord. This network was of greater complexity and density in the gray matter than in the white. From the gray matter, His' injections passed outward in channels around the blood-vessels to spread beneath the pia in a large subpial network. Since this time, the existence of these perivascular channels has been generally accepted, although for a while they were assailed as artifacts. Whether these channels are lined by mesothelium is not definitely established; it seems most likely that the pial mesothelium runs inward with each vessel to form an outer layer of the space. The inner wall of this tube is probably similarly derived from the mesothelial covering of the vessels, which are thus protected throughout the subarachnoid spaces. This cellular covering of the perivascular tube seemingly continues inward only a short distance, neuroglia cells probably replacing on the outer surface the mesothelial elements.

The perivascular spaces connect, according to all of the best observations, with the subarachnoid spaces (His' finding of a subpial termination not being verified). Not only have many pathological findings substantiated this connection of the perivascular spaces, but physiological and toxicological evidence has aided in establishing this conception. Milian ('04), in a case of subarachnoid hemorrhage, found the perivascular system filled with blood, but the most striking demonstration of the subarachnoid connections of these spaces was made by Spina ('98, '99, '00 a, b). This worker found a punctate exudation of a clear impid fluid from the exposed surface of the brain, following great rises in systemic arterial pressure and subsequent rises in intracranial tension. Lewandowsky ('00), accepting

Spina's evidence as conclusive, hypothesized that the cerebro-spinal fluid was really the lymph of the central nervous system and was the product of the activity of the nervous tissue, poured into the subarachnoid spaces.

Mott's important observations ('10) of a great dilatation of all the perineuronal and perivascular spaces, after ligations of certain of the head arteries, offer strong anatomical evidence of the subarachnoid connections. In these cases of cerebral anemia, the dilated spaces about the nerve-cells were found to be directly connected with pericapillary spaces and these in turn were joined to the perivascular channels and the subarachnoid spaces. Mott hypothesized from these findings a drainage of the cerebro-spinal fluid from the subarachnoid spaces into the cerebral capillaries by way of the perivascular channels. The flow of the fluid, then, on such a basis would be from the subarachnoid spaces into the nervous tissue—toward the capillary bed.

In an investigation of this subject (Weed, '14 c), spinal subarachnoid injections of an isotonic solution of foreign salts were made. The salts were subsequently precipitated as insoluble prussian-blue, and hence the course taken by the solution could be verified. In the routine replacement of the normal fluid or in an injection under pressures but slightly above normal, the subarachnoid spaces, in the course of a few hours, were found to be filled with the precipitated injection-mass, but practically none of this appeared in the perivascular system. In similar injections but under pressures of about 50 mm. Hg, the perivascular spaces were sometimes filled with the foreign salts, but only to the pericapillary spaces. To secure a really complete filling of the perivascular and pericapillary spaces, a special procedure was found necessary: the subarachnoid pressure was maintained at normal by the continued injection of the isotonic foreign solution and subsequently a cerebral anemia was caused. In such a case, the experimental cerebral anemia caused a decrease in the intracranial fluid contents sufficient to cause an aspiration of the foreign solution from the subarachnoid spaces (the cranium being potentially a closed box) and to fill the perivascular system. The nerve-cells in such a case were surrounded

by a thin collection of minute prussian-blue granules; these could be traced through pericapillary and perivascular spaces to the subarachnoid spaces.

From an analysis of these results, it was concluded (Weed, '14 c) that the fluid current in the perivascular system was from nerve-cell to subarachnoid space and that by this way a small addendum of cerebro-spinal fluid drained into the meningeal spaces. This view had already on rather insufficient grounds, been advanced by Plaut, Rehm and Schottmuller ('13) and by Mes-trezat ('12). Frazier ('15) has since accepted the view of a double source of the fluid, and Halliburton ('16) inclines to a consideration of the fluid as the "lymph of the brain" but only in a restricted sense.

Such a conception of a dual source of the cerebro-spinal fluid has received support from other observations than those recorded in the foregoing paragraphs. Jacobson's important chemical studies (unpublished) have demonstrated a distinct difference between the subarachnoid fluid (product of chorioid plexuses and perivascular system) and the ventricular fluid (product of chorioid plexuses alone). The former fluid is richer in protein and poorer in sugar than the latter—a finding to be expected if the products of nerve-metabolism are poured into the subarachnoid space. Likewise, distinct serological differences between subarachnoid and ventricular fluids from the same patient have been reported. And pathologically the occurrence of intracortical cysts from dammed-up perivascular channels indicates strongly a production of fluid within the nervous tissue itself, draining outward into the subarachnoid spaces.

THE ABSORPTION OF THE FLUID

Modern anatomical evidence regarding the absorption of cerebro-spinal fluid was first brought forward by Key and Retzius ('76) in an epochal monograph. These investigators injected gelatin solutions colored with Berlin blue into the spinal subarachnoid spaces of a cadaver. The pressures used were somewhat excessive (about 60 mm. Hg), but the continuity of spinal and cranial subarachnoid spaces was demonstrated beyond

doubt. The gelatin was also found to give evidence of a direct escape of fluid from the cranial subarachnoid spaces into the great venous sinuses of the dura through the Pacchionian bodies. In addition to this major blood-vascular absorption, a lesser drainage of the fluid into lymphatic vessels was indicated; this drainage was not direct but apparently through perineural spaces around certain of the cranial nerves.

Quinke's work ('72), though published somewhat before the monograph of Key and Retzius appeared, did not antedate their earlier reports. The greater part of Quinke's data was based on the results obtained by injecting cinnabar into the spinal subarachnoid spaces and, after varying periods, studying the meninges of the animals. Quinke found these red granules rather uniformly distributed throughout the spinal region and lodged chiefly in the basilar portion of the cranium; in both regions the granules were wholly in the subarachnoid spaces. But the granules of cinnabar were held largely by phagocytic cells; this was particularly the case in the structures along the venous sinuses which he termed the Pacchionian granulations. Quinke also identified the sulphide in the cervical lymph-nodes; here again it was held in the cell-bodies of lymphocytes.

For several years after Key and Retzius, this view of the absorption of the cerebro-spinal fluid endured, but gradually with the realization that the Pacchionian granulations, as such, do not exist in infants and in the lower animals, it was felt that this view was inadequate. For the next two decades practically nothing was published on this subject; then rather suddenly considerable work of a physiological nature was done. Most of this was planned to demonstrate the venous or lymphatic drainage of the cerebro-spinal fluid. But the anatomical data included in these observations were not of great importance.

Reiner and Schnitzler ('94, '95) injected salt solutions containing potassium ferrocyanide into the spinal subarachnoid spaces and recovered the ferrocyanide from the jugular vein in from thirty to forty seconds after the injection—physiological proof of a rapid blood-vascular absorption. Similarly, though with a somewhat slowed stream, olive oil was recovered from the

jugular vein. These investigators state that, as Pacchionian granulations do not occur in the animals used, other pathways of absorption for the cerebro-spinal fluid must exist.

Following Reiner and Schnitzler, a number of workers offered physiological evidence of the pathways of absorption of cerebro-spinal fluid. Leonard Hill ('96) was able to trace saline solution colored with methylene blue "straight into the venous sinuses" after spinal injection. While the abdominal viscera were colored with the blue within a few minutes, the cervical lymphatics were not colored until after one hour. Hill found also that serum apparently passed into the sinuses as readily as did saline. Ziegler ('96) observed that, after introduction of potassium ferrocyanide into the cerebro-spinal fluid, the substance could be identified in ten seconds in the vena facialis posterior and only after thirty minutes in the cervical lymph channels. Similarly Lewandowsky ('00) identified sodium ferrocyanide in the urine of animals within thirty minutes after intraspinal injection.

Although Spina's ('98, '99, '00) experiments may be adversely criticized because of the employment of excessive pressure, they still offer collateral evidence of great value in regard to the drainage of cerebro-spinal fluid. This worker introduced fuchsin solutions into the subarachnoid spaces of animals, but recently killed or under anesthesia, and ascertained that the venous absorption was by far the more important. He further found that with increasing pressures of injection the lymphatic channels function the more readily and carry away a great proportion of the fluid.

This idea of a greater venous absorption of the fluid was also brought forward by Cushing ('02 a, b), in the course of a study of the effects of local and general cerebral compression. After subdural rupture of a mercury-filled rubber bag, used to secure local compression, Cushing found the mercury globules in the great dural sinuses, in the diploetic vessels, in the jugular veins and in the right heart, but none was present in the cervical lymphatic chain. Cushing's observations of a direct passage of mercury, from the spinal subarachnoid space of a cadaver, directly into the superior sagittal sinus are quite analogous.

With intraspinous injection of non-absorbable gases, the same venous channels could be made out, the gaseous bubbles being apparent in the jugular veins, but not in the cervical lymphatics. From these experiments, Cushing hypothecated other channels than the Pacchionian granulations as the functionally active pathway of escape for the cerebro-spinal fluid, and suggested a valve-like mechanism of fluid-passage between the subarachnoid spaces and the great dural sinuses. Recently ('14) he has adopted a different view.

Several other views have been advanced by workers in this field. Mott ('10), from a study of the brains of monkeys in which an experimental anemia had been caused, suggested that the cerebro-spinal fluid was absorbed in the cerebral capillaries by way of the perivascular system. Assuming that this fluid was similar to other body-fluids, Mott conceived that by diffusion and osmosis the blood in the cerebral capillaries would receive water and carbon dioxide from the cerebro-spinal fluid in the pericapillary spaces and in return the blood would give salts and sugar to the cerebro-spinal fluid. Mott's chemical observations recorded a high content in the cerebro-spinal fluid of carbon-dioxide—a finding which would seemingly suggest that the fluid had received already these products of metabolism. Dandy and Blackfan ('13) likewise consider the drainage of cerebro-spinal fluid "a diffuse process from the entire subarachnoid space," basing their conclusions upon the results of subarachnoid injections of phenolsulphonephthalein. Their results with this solution with intact cerebro-spinal spaces coincide with those given above for the absorption of true solutions; with granular material (india ink) there was found practically no absorption from these channels—a finding which coincides with the results obtained by Quincke.

The idea of an absorption of cerebro-spinal fluid solely by way of lymphatic vessels has been developed by Cathelin ('12) in a recent monograph. He has rather sweepingly condemned all of the preceding work indicating a major venous drainage, defending on insufficient grounds his contention of a sole lymphatic drainage by way of the perivascular and perineural sheaths.

Goldmann ('13) likewise inclines somewhat to the idea of lymphatic absorption, basing his conception on the staining of cervical lymph-nodes after intraspinous injection of trypan blue. While acknowledging the limitations of his method and the weight of evidence in favor of venous absorption, Goldmann points out that much of the earlier work is valueless because of failures to control pressures.

With this somewhat contradictory work as a basis, an investigation was undertaken (Weed, '14 a, b, c) to determine if possible the actual pathways by which the cerebro-spinal fluid was returned to the general blood-stream. It was felt that most of the observations already made were not conclusive as demonstrating anatomical pathways, for the morphological studies reported had all been made after injections of viscous colloidal fluids (gelatin) or of suspensions of granular material (cinnabar, india ink). The physiological findings after injections of true solutions (methylene blue, potassium or sodium ferrocyanide, phenolsulphonaphthalein, fuchsin, etc.) offered much more reliable evidence of a major venous absorption, but no anatomical pathways had been demonstrated. After critical analysis of the methods used and the criteria necessary for reliable results, true solutions, isotonic with the body-fluids were injected, under pressures but slightly above the normal, into the spinal subarachnoid spaces of anesthetized animals. A solution of foreign salts, potassium ferrocyanide and iron-ammonium citrate, in a 1 per cent concentration was used; the course of this solution could subsequently be histologically traced if the tissue was fixed in toto in an acid medium, precipitating prussian-blue. In addition to these injections at low-pressures in the anesthetized animals, simple replacements of the normal cerebro-spinal fluid by the foreign solutions, without altering the normal tension, were made. These injections under low pressures were continued usually for several hours to secure complete filling of the subarachnoid channels; after a replacement, the animals were kept alive for about the same length of time. The ferrocyanide-citrate solution proved of great value as it was not attracted to any specific cell-elements and as it was non-toxic

within the subarachnoid spaces. In this way, a physiological method of ascertaining an anatomical pathway was employed; alterations in the method were made to investigate certain phases of the problem.

From this work (Weed, '14 a, b, c) certain apparently definite results were brought forth. Accepting as evidence of the normal pathway the course taken by the solution (as determined by the resultant precipitate of prussian-blue) in the procedures approaching the physiological, it was found that the solution rather rapidly spread through the subarachnoid spaces of the spinal cord. In the cranial cavity, the injection first passed into the basilar regions (in agreement with Quincke and Goldmann) and thence slowly filled the subarachnoid spaces over the cerebral hemispheres. There was no penetration anywhere of the mesothelial cells enclosing the subarachnoid spaces, showing them to be impenetrable to this foreign true solution—an important feature in the reliance placed on the results obtained by this method. The fluid was absorbed directly into the venous sinuses by way of the arachnoid villi, which have already been described in a foregoing section of this communication. In these structures, the precipitated granules could be traced directly from the cerebral subarachnoid spaces; they were accumulated in the delicate core of the villi. The foreign solution (as evidenced by the granules) had left the villi by passing through the mesothelial cells of the arachnoid and also through the endothelial cells of the venous sinuses, thus demonstrating a direct absorption into the dural sinuses. The passage into the venous stream seemed largely a matter of filtration from a point of higher pressure (subarachnoid space) to a point of lower pressure (venous sinus), although the factors of diffusion and osmosis were not absolutely ruled out. In this connection the observations of Wegefarth ('14 b) and also of Dixon and Halliburton ('14) regarding cerebro-spinal pressures are of interest. It was found also that the absorption of true solutions from the cranial subarachnoid spaces was a much more efficient and rapid process than was the corresponding absorption from the spinal region. Suspensions of particulate matter, however, were invariably

found not to pass into the venous sinuses but to lodge largely in the meshes of the subarachnoid spaces and in part in the arachnoid villi.

In addition to this major venous absorption through arachnoid villi directly into the great dural sinuses, an accessory drainage by way of the lymphatic system was described, the observations being based on the same method of experimentation (Weed, '14 b). This is a much slower, less efficient means of escape for the cerebro-spinal fluid, normally caring for but a small fraction of the total drainage in all probability. This lymphatic absorption is wholly indirect; the fluid reaches the true lymphatic vessel only outside of the dura and of the cranium. The mechanism for this drainage is by way of perineural spaces around the spinal nerves to a slight extent but chiefly around certain of the cranial nerves—particularly the olfactory branches. The precipitated prussian-blue (evidence of the distribution of the foreign true solution) could be made out in a perineural arrangement around the olfactory branches in the nasal membrane; the granules escaped into the interstitial tissue and from this the absorption into the lymphatic vessels took place. On the course of certain of the cranial nerves (optic, acoustic, etc.) such an indirect, secondary lymphatic drainage apparently does not occur.

Almost simultaneously with this publication, Frazier and Peet ('14) reported the results of subarachnoid injections of methylene blue, isamine blue, trypan red and blue, and phenol-sulphonephthalein. The trypan blue did not reach the lymph-glands of the neck until after several hours, but the phenolsulphonephthalein was detected in the torcular blood in from two to three minutes. They regarded the blood-vascular absorption as most important and the lymphatic drainage as a minor accessory mechanism.

Since these more recent observations were issued, Dixon and Halliburton ('16), in the course of their excellent studies on cerebro-spinal fluid, have reported the results of their physiological experiments on the absorption of the fluid. They found, in confirmation of the results given above, no absorption of particulate matter and a free and rapid absorption of true

solutions. Between the two types, they discovered a much slower absorption of colloidal solutions, the larger molecules being absorbed more slowly than the smaller. This report represents a very valuable contribution to the subject of absorption. Furthermore, Dixon and Halliburton agreed that the spinal portion of the subarachnoid spaces did not possess great powers of absorption as compared with the cranial drainage, which was much more efficient. This observation, in accord with previous work, rendered the hypothesis of Dandy and Blackfan ('13) untenable. Likewise it casts perhaps doubt on the possibility of Mott's theory ('10) being correct, for it is difficult to assume a power of absorption of cerebro-spinal fluid by the cerebral capillaries without granting a similar function to the spinal. For other reasons, however, these theories of drainage seem insufficient (Weed, '14).

Dixon and Halliburton, moreover, could not find, during the period of observation, evidence of any lymphatic absorption by tapping the thoracic duct; the whole absorption seemingly went by way of the blood-vascular system. They conclude, as does also Halliburton ('16) that "the fluid probably reaches the venous sinuses by way of the microscopic arachnoid villi." Their findings in regard to the lymphatic absorption are probably to be accounted for by the very slow process of drainage along this pathway.

Thus it seems fair to assume, from the evidence presented above, that the absorption of cerebro-spinal fluid is a dual process, being chiefly a rapid drainage into the great dural venous sinuses, and in small part, a slow escape into the true lymphatic vessels, by way of an abundant but indirect perineural course.

EMBRYOLOGY OF THE CEREBRO-SPINAL SPACES

With this conception of the processes of the cerebro-spinal fluid apparently indicated by the investigations up to the present, the embryology of the pathways for this fluid will be discussed. At the Christmas meeting of the American Association of Anatomists in 1915, the results of replacing, in living pig-embryos, the existing ventricular fluid by a foreign true solution

were presented (Weed, '16 a). The method of replacement provided for the exchange of fluids without increasing the intraventricular pressure, and the embryos were subsequently kept alive for an hour or so, the foreign solution being non-toxic and not attracted to specific cellular elements. In this work, an isotonic solution of potassium ferrocyanide and iron-ammonium citrate was used; these salts were precipitated subsequently as prussian-blue and the resultant granules were considered to represent the course of the normal cerebro-spinal fluid.

By this method of investigation, it was found that in pig-embryos up to about 14 mm. in length, the replaced true solution remained wholly within the central canal of the spinal cord and within the cerebral ventricles. In the smaller stages (about 8 mm.) the injection showed no peculiarities but in a somewhat larger stage (13 mm.) a definite, very dense oval of precipitated prussian-blue could be made out in the central portion of the roof of the fourth ventricle. It was through this oval in the rhombic roof that the fluid passed from the cerebral ventricles into the extraventricular tissues, in the stages in pig-embryos of over 14 mm. This extraventricular spread of the fluid indicated that the balance between the intraventricular production of fluid and the increasing volume of the cerebral ventricles was destroyed; it was of considerable interest that this overthrowing of the balance should occur coincident with the development of tuftings in the chorioid plexuses of the fourth ventricle. From this stage of the initial outpouring of fluid from the cerebral ventricles, the further spread was not extensive until a length of about 18 mm. was reached in the pig-embryo. At this older stage, two areas, one in each half of the now divided rhombic roof, affording means of fluid-passage from the ventricles, were made out. From both of these areas the fluid escaped into the perimedullary tissues in exactly the future subarachnoid arrangement. In pig-embryos of over 18 mm., the further extension of the pathways of the embryonic cerebro-spinal fluid occurred rapidly. At 19 mm., the peribulbar tissues had become filled with the fluid; and within a couple of millimeter's growth, the whole perispinal spaces were invaded by the fluid, the de-

velopment apparently being from above downward. At the same time, the basilar mesencephalic region was found filled by the fluid and an infundibular extension very quickly occurred. At a stage of about 26 mm., a complete perimedullary distribution of the replaced fluid was recorded—the arrangement of fluid at this period being that of the adult.

This escape of fluid from the roof of the fourth ventricle into the extraventricular tissues occurs through two areas of ependymal differentiation (Weed, '16 a). The superior of these areas makes its appearance as a definite oval in the roof of the fourth ventricle (cf. replacement injection of stage of 13 mm.) and represents an earlier differentiation of the original ependymal lining of this ventricular cavity. Quite similarly, as this oval comes to lie in the superior half of the rhombic roof after this structure is transected by the laterally developing chorioid plexuses, a second area of differentiation of the ependyma develops in the inferior half of the roof. The superior area reaches its maximal anatomic differentiation and apparent functional significance at a stage in the pig of 19 to 20 mm.; from this point on, it undergoes a regression, sacrificed to the greater development of the chorioid plexuses and the downward growth of the cerebellum. The inferior area, however, persists as a functional area of fluid-passage, gradually occupying the whole inferior velum chorioidea. Whether a permanent persistence of this area during adult life occurs, cannot be answered until more data regarding the foramen of Magendie are at hand. The differentiation of the ependyma in these areas is largely a transformation to a flattened, elongated type of cell. Through the cell-cytoplasm in this area, as through a cellular membrane, fluid passes in accordance with the laws of filtration and possibly of diffusion (Weed, '17).

A year after the work detailed above was presented, Keegan ('17) reported in an abstract, the results of a similar investigation, carried out independently. Keegan made use of the same isotonic solution of potassium ferrocyanide and iron-ammonium citrate, and also of a solution of the citrate alone. In the main, Keegan's results were similar to those given above, particularly

in regard to the occurrence of two areas of ependymal differentiation in the roof of the fourth ventricle. Instead of making use of replacements, he employed a finely drawn glass cannula and made injections directly into the lateral ventricle of living embryos. Such a method, because of the unavoidable increase in the intraventricular pressure, hardly seems to offer results of as great reliability as are afforded by the replacement method. Keegan reports that the injection of the double solution in living rabbit embryos did not undergo any extraventricular spread until a stage of 17 days was reached, although the specimens showed an oval aggregation of granules in the rhombic roof; solutions of the iron-ammonium citrate alone, however, gave early extensions through the roof of the ventricle. Similar results were obtained in chick-embryos, the roof being impermeable to the double solution and permeable to the citrate alone. It seems rather difficult to understand this relative impermeability of the rhombic roof to the combined solutions of the citrate and ferrocyanide; this combination has been used with success by several investigators during the last few years, in many problems of absorption through membranes. The reactions of the rabbits and chicks may be such as to prevent such fluid-passage. After injection with the citrate solution, Keegan records an extraventricular spread shortly before the chorioid plexuses develop; this is somewhat at variance with the results in pig-embryos after replacements (Weed, '16 a).

The cerebro-spinal fluid of the embryo, passing from the cerebral ventricles, is poured into the perimedullary mesenchyme (Weed, '16 b). This mesenchyme about the central nervous system in the early stages (about 10 mm. in the pig) is a loose tissue characterized by a somewhat small mesh, with frequent oval nuclei; the cytoplasm is largely prolonged into the syncytial formation. As soon as the embryonic ventricular fluid enters this tissue, a differentiation is begun. This changing process begins in the peribulbar tissues and spreads thence both downward about the spinal cord (here the process is almost synchronous with the peribulbar) and more slowly upward about the cerebral hemispheres. This differentiation consists of a modification of the primary small-meshed mesenchyme into

the larger arachnoid spaces. Thus, with the initial outpouring of fluid, a process of dilatation of the perimedullary mesenchyme begins; the spaces between the syncytial strands seem first to become enlarged and filled with a more albuminous fluid (as evidenced by the resultant coagula). Subsequently with further differentiation, some of the cytoplasmic strands are broken off; the processes recede and the whole cell-body may become merely an integral part of a persisting, much strengthened strand—a future arachnoidal trabecula. In certain areas, as in those of the future cisternae, the process of breaking down of the mesenchymal mesh and the formation of new arachnoid spaces reaches its maximum, for here the spaces become relatively very large and the number of trabeculae correspondingly fewer.

Associated with this differentiation of the perimedullary mesh into the larger arachnoid spaces, there occurs at the same time a process of mesenchymal condensation. Such a thickening becomes evident not only in the development of the persisting arachnoidal trabeculae, but chiefly in the formation of the outer continuous arachnoid membrane. This first appears as a thin zone of secondary condensation, between the enlarging spaces of the perimedullary mesenchyme and the thickening blastema of the bony coverings. In this way, the perimedullary mesenchyme is subdivided into two zones as described by His ('65 b) and Kölliker ('79). Farrar ('06) has also recorded in a brief note a somewhat similar division of the mesenchyme in the chick. However, this zone of condensation represents not only the arachnoid membrane (as distinguished from trabeculae) but also the inner surface of the dura. In this narrow thickening of the mesenchyme, differentiation occurs, permitting a cleavage of the membranes (50 mm. in the pig). Thus, in the formation of the arachnoid there occur two processes—a dilating, enlarging process forming the subarachnoid spaces, and a condensing, thickening process resulting in the formation of the arachnoidal trabeculae and membrane.

While the mesenchymal dilatation in the perimedullary spaces is proceeding, the pia mater is being formed out of the same mesenchymal elements. At first, with the extensive capillary

plexus investing the nervous system, the cells of the mesenchyme form apparently a single and very often a double layer about the neuraxis, in an adult pial relation. The breaking down of the original small mesenchymal mesh still leaves a layer of pial cells closely attached to the nervous tissue; these are continuous with the cells covering the arachnoid trabeculae. Some of these cells also must be considered as invading the nervous tissue to some extent to form lining elements for the perivascular spaces. This embryonic pia is a comparatively efficient membrane, resisting forces from within (as shown by the occurrence of subpial extravasations after experimental rupture of the nervous system from excessive injection pressures) and being impermeable to solutions from without (as in the subarachnoid extensions of replaced foreign solutions).

The mesenchymal cells, enclosing the newly formed subarachnoid spaces and also covering on its outer surface the arachnoid membrane, undergo a rather gradual differentiation into the low cuboidal mesothelial elements which in adult life cover these fluid-spaces. A few of the mesothelial cells, both in the trabeculae and in the membrane, are devoted finally to the formation of the meager supporting tissues of these structures. The differentiation of pial cells is wholly similar to the process in the so-called arachnoidea, for this pial membrane both embryologically and functionally is merely the inner retaining layer of the subarachnoid channels.

After the appearance of the secondary condensation of mesenchyme, separating the perimedullary tissue into two zones, the formation of the dura may be traced. In the outer zone, extending to the blastemal condensations, a distinct increase in the number of mesenchymal elements may be made out; all of this outer zone of tissue is ultimately devoted to the formation of the fibrous portion of the dura. The process begins in the basilar regions of the cranium and spreads upward, following somewhat the differentiation of the arachnoid spaces and of the developing cranium. Fibrous tissue may soon be detected in this denser zone and the gradual formation of a dense fibrous membrane may be made out. The inner surface of the dura,

however, develops in connection with the outer surface of the arachnoid membrane; these together are included in the secondary zone of condensation. As soon as a distinct separation of the two membranes over the cerebral hemispheres can be made out in fetal pigs (50 mm.), a polygonal mesothelial cell-pattern on the inner surface of the dura may be demonstrated by silver reductions.

A combined study of the processes of dilatation of the mesenchymal spaces and of specimens prepared after replacements of the embryonic ventricular fluid by the ferrocyanide solutions, shows that the mesenchymal cells are impermeable to this true solution, that both the pial cells, and the secondary condensation present barriers to the spread of the fluid, and that the course taken by the replaced fluid coincides with the thinning of the arachnoid mesh and with the presence of an increased amount of the albuminous coagula (suggesting the presence of the protein-rich embryonic fluid). Thus the anatomical limits of the spread of the foreign solution coincide apparently with the physiological use of these fluid-channels.

A full account of the embryology of the cerebro-spinal spaces is being published as one of the Contributions to Embryology of the Carnegie Institution of Washington (Weed, '17). In the account given above, only the more general processes have been presented; for the evidence and more complete information, reference to the Carnegie publication must be had.

DISCUSSION

The processes, then, of the cerebro-spinal fluid are in many respects fairly well established. The evidence for the current beliefs is extremely good for certain viewpoints concerning the anatomical pathways involved and for certain physiological phenomena. When, however, the viewpoint here expressed is compared to the accounts appearing in the current text-books and in some of the current literature, a marked diversity of opinion is brought forth.

It does, however, seem established, on definite and firm grounds, that the cerebro-spinal fluid is largely produced within

the cerebral ventricles by specially differentiated structures, the chorioid plexuses. After traversing the ventricles, this fluid passes from the fourth ventricle into the subarachnoid spaces; these are channels adapted for fluid-passage and permit the distribution of the fluid everywhere about the central nervous system. From these spaces, the fluid returns to the blood-vascular system, the mode of absorption being a process largely of filtration and of diffusion through arachnoid villi into the dural sinuses.

But in addition to the production of the cerebro-spinal fluid by the chorioid plexuses, there is apparently a further addition of fluid by way of the perivascular system. This is evidently derived primarily from the capillaries of the central nervous system; it passes from these structures directly into the pericapillary spaces and thence to each nerve-cell or outward through the perivascular channels into the subarachnoid spaces. The amount of fluid derived by this method is undoubtedly small; it is presumably somewhat different in its chemistry from the fluid elaborated by the chorioid plexuses. There is, likewise, an accessory mechanism for the absorption of fluid, in addition to the major drainage into the venous sinuses of the dura. This minor method of escape for the cerebro-spinal fluid is by way of the perineural spaces (particularly those of certain of the cranial nerves) indirectly into the lymphatic system. This is, according to the best evidence, a slow mechanism; it probably cares for but a very small portion of the total absorption of fluid.

It might well be conceived that much of the confusion regarding the relations of the cerebro-spinal spaces to the lymphatic system has had its origin in such a conception of an accessory method of production of cerebro-spinal fluid and in this indirect absorption by the lymphatics. This view, however, does not include, wholly, the real explanation of the difficulty about the lymphatic relationship; the idea of lymph channels in the dura and in the leptomeninges was advanced many years before the problems of production and absorption of the cerebro-spinal fluid were really modernized.

This older view that the meninges themselves possessed lymph vessels was presumably based chiefly on the work of Arnold ('38) who described three definite layers of lymphatic vessels in the pia-arachnoid. This work has been quite widely quoted, due to His' ('65, a) somewhat comparable findings about thirty years afterward. His was able to make out a definite subpial plexus of supposed vessels after intramedullary puncture-injection—a method which today would hardly be relied upon for such an investigation. In the dura, Böhm ('69), by similar puncture-injection with Berlin blue, demonstrated an apparent spread of the foreign mass into definite channels resembling the network of other parts of the body.

The evidence given by these investigators probably represents the best offered for the definite occurrence of lymphatic vessels within the meninges. It was brought out before the lymphatic system was definitely found to be a series of closed tubes and before the more modern criteria of lymphatics were established. Puncture-injections should yield a definite plan of lymphatic network, substantiated by histological evidence of an endothelial channel. But in the leptomeninges, puncture-injections or extensions from other injections, show a peculiar network—not lymphatic but representing merely the filling and distension of tissue-channels in these membranes. In the pachymeninx, likewise, puncture-injections outline a definite network, resembling only superficially a lymphatic plexus. This was reported by Böhm ('69) using Berlin blue and was also observed in several cases on human dura after india ink injections (Weed, '14c). But the channels in the dura, injected by this method, are not lymphatic vessels; they are not lined by endothelium nor is the pattern of the network typical in any way of a lymphatic plexus. These dural channels are merely unlined spaces between the dense strands of fibrous tissue; similar unlined spaces may be demonstrated by similar injection in any dense fibrous membrane.

But in the dura there is another factor which contributes to the possible confusion. This is the occurrence in that membrane of definite columns of cells, differentiated clearly by their

morphology and tinctorial reactions from the dural tissue. These cell-columns, often simulating to some extent a vascular endothelial tube, could be confused with lymphatics, but all of the evidence demonstrates that these cell-columns are arachnoidal in origin, representing intradural prolongations of the arachnoid cells and forming a potential channel for cerebro-spinal fluid. They are primary pathways related to the veins and are not secondary or lymphatic.

The more recent work, making use of the more definite criteria for lymphatic vessels and excluding rigorously tissue-spaces or other potential channels, has not established the occurrence of definite endothelial-lined lymphatic vessels in the three meninges. Such a view receives great support from the work of Sabin ('12) who has never observed, in many injections of the head region in embryos, any extension of lymphatic vessels into the dura. Likewise, all recent anatomical work on these perimedullary membranes in adults has failed to demonstrate any lymphatic channels. Hence it seems essential at the present time to conclude that there occur, in the meninges, no true endothelial-lined lymphatic vessels. It is, however, obvious that evidence of such a negative character is more likely of future contradiction than analogous positive demonstrations. Every argument, though, points to an avoidance of the meninges by the developing lymphatic sprouts.

If then, there is a definite lack of lymphatic vessels in the meninges, there must necessarily be a similar lack of these vessels in the nervous tissue itself. This view is very definitely established in every way; the true endothelial-lined lymphatic vessels do not occur in the cerebro-spinal axis. With a lack of these vessels in the meninges it would be very difficult to assume that any could occur in the central nervous system. In this connection, it must be emphasized that the subpial plexus of His has not been confirmed; all the evidence is against the acceptance of such a conception. In the place, possibly, of the true lymphatic vessels, there occur in the nervous tissue the perivascular spaces. These partially lined channels have in the past been termed the 'perivascular lymphatic spaces' or

'perivascular lymphatics' but in more recent publications, the 'lymphatic' has been omitted, so that the spaces seem best termed 'perivascular' spaces. Streeter ('17) has very recently modified likewise the terminology of the spaces about the ear, terming the perilymphatic spaces the 'periotic.'

These perivascular channels, according to the data presented in a foregoing section of this paper, pour into the subarachnoid spaces a small amount of fluid. It represents probably the medium of fluid-exchange between the blood-capillary and the nerve-cell, the excess of this intramedullary fluid being eliminated by the perivascular system. It subserves, then, in a way, the function of the tissue-fluid of other regions and tissues of the body, being analogous to the other tissue-fluids but lacking the possibility of direct absorption by the lymphatic system. This intramedullary fluid is, in this restricted sense, an 'extra-vascular lymph' or, as Halliburton terms it ('16), "the lymph of the brain." But such a conception of the function and distribution of this intramedullary tissue-fluid in no way justifies the application of the word 'lymph' to the cerebro-spinal fluid. For it does seem essentially important to continue to regard the fluid existing within the true lymphatic vessels as being the only 'lymph.' Tissue-fluids (or extravascular lymph, a poor term) in most of the other parts of the body have a definite and close physiological relation to the lymphatic vessels; here in the central nervous system, the tissue-fluid from the nerve-cells represents merely a small addition to the cerebro-spinal fluid already traversing the subarachnoid spaces. Thus, as no true endothelial-lined lymphatic vessels apparently exist within the meninges or central nervous system, the cerebro-spinal fluid cannot be regarded as 'lymph.'

In many other respects, the processes of the cerebro-spinal fluid exclude it from the designation of 'lymph.' The anatomical characteristics of its pathway—at first, with an ependymal lining and subsequently, with a low, cuboidal mesothelium—surely separate this system from the lymphatic vessels. The embryological formation of the cerebro-spinal spaces is quite different from the origin and development of the lymphatic

system (cf. Sabin '13 a, b). For here in these intramembranous channels, there occurs a dilatation and breaking down of the original mesenchymal tissue-spaces to form the final subarachnoid spaces. This is a process entirely apart from our present conceptions of a centrifugal growth of a lymphatic system from a venous endothelial origin. In these perimedullary spaces, the developmental arrangement has no primary vascular relations; it follows apparently the physiological use of these spaces as fluid-channels. This mode of formation of the cerebral-spinal spaces is quite similar to the process which results in the formation of the anterior chamber of the eye. In this aqueous reservoir, however, the destruction of the mesenchymal syncytial strands becomes complete (except in the spaces of Fontana), a phenomenon which has its direct analogy in the formation of the subarachnoid cisternae.

The analogies between the processes of the cerebro-spinal fluid and of the aqueous humor are very marked and very numerous (Henderson ('10), Wegefarrh and Weed, ('14)). The fluid in both eye and brain is chemically identical; it is in both places produced by similar epithelial structures—the ciliary processes and the choroid plexuses. The fluid first traverses epidermal-lined structures—posterior chamber and cerebral ventricle—passing through foramina into the mesodermal spaces (anterior chamber and subarachnoid spaces). The major absorption in both cases is into venous sinuses (canal of Schlemm and great dural sinuses) through the mechanism of cellular prolongations, the pectinate villi in the eye (Wegefarrh, '14a) and the arachnoid villi (Weed, '14b). The accessory production of fluid is likewise probably of the same character in the two organs.

Thus in many ways, the cerebro-spinal fluid must be looked upon as being a characteristic body-fluid, quite apart from the ordinary tissue-fluids and from the true lymph of the endothelial-lined vessels. It may be considered identical with the aqueous humor (and possibly with the fluid of the periotic spaces) and may be likened, as Halliburton ('16) has suggested, to a physiological salt solution. In only a very restricted and minor sense may it possibly be referred to as lymph; may not this usage of the term be definitely discarded?

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日本解剖學者及動物學者諸君

謹啓現下歐洲ニ於ケル不幸ナル戰禍ハ各方面ニ少ナカラヌ影響ヲ及ボシ候ガ就中科學的研究ハ多大ナル阻碍ヲ蒙リ歐洲ニ於ケル解剖學及動物學ニ關スル幾多ノ雜誌ハ或ハ發行中止ノ己ムナキニ至レルモノアリ然ラザルモノモ之ヲ米國ニ於テ手ニ入ルコト甚ダ困難ナル狀態ニ有之申候 就テハ

形態學雜誌、比較神經學雜誌、解剖學雜誌、解剖學彙報、實驗動物學雜誌、五雜誌ノ編輯局ハ此時局ニ際シテ及ブ限り右學術ノ研究ニ對シ補助ト獎勵トヲ與ヘシ希望ヲ以テ世界各國研究所ノ業績ニ對シ廣ク出版上ノ便宜ヲ斗ルコトニ相成申候即チ解剖學及動物學上ノ諸論文ハ其編輯當局ノ認許ヲ得次第迅速ニ英、佛、獨、伊及西班牙語ノ何レニテモ登載可仕候 從來上記ノ五雜誌ハ生物學ニ關スル米國研究家ノ業績ノ大多數ヲ網羅シ且世界各國ニ最モ廣ク汎布致サル有カナル雜誌ニテ孰レモ米國貴府ウイスター研究所ガ斯學推輓ノ目的ニテ出版致シ居ルモノニ有之其編輯局ハ米國及加奈太ノ重ナル代表的研究家ノ殆ド凡テヲ包含致居候 就テハ今回右編輯局ハ日本帝國ニ於ケル解剖學及動物學者ノ御便宜ノ爲メ其業績ヲ右五雜誌ニ於テ發表セラレンコトヲ切望致候而テ之ニ依テ將來日米兩國研究者間ノ親交ヲ幫助スルコトヲ得バ至幸實ニ之ニ過ギスト存候 尚出版ニ關スル凡テノ御照會ハ何卒尤記ヘ宛テ御文通被成下度願上候 敬具

北米合衆國ペンシルヴェニア州フィラデルフィア市

ウイスター解剖學及生物學研究所所長ドクトルミルトン・ジ・グリーンマン

A causa de las lamentables condiciones por que atraviesa Europa, en algunos de los países más importantes del mundo se han interrumpido seriamente los trabajos de carácter científico, y una gran cantidad de revistas anatómicas y zoológicas han suspendido su publicación, mientras que otras se han vuelto más o menos inaccesibles para los investigadores, y con especialidad para los investigadores que trabajan en los laboratorios americanos.

Deseosos de ayudar y alentar en lo posible las ciencias anatómica y zoológica, y a los sabios que a ellas se dedican, los editores de

Journal of Morphology,
Journal of Comparative Neurology,
American Journal of Anatomy,
Anatomical Record y
Journal of Experimental Zoology

ofrecen las columnas de estas publicaciones a los laboratorios de todo el mundo.

Los estudios anatómicos y zoológicos que sean aprobados por los editores, recibirán inmediata publicación en inglés, francés, alemán, español o italiano.

Estas publicaciones contienen abundante material de investigaciones hechas en América en asuntos de biología, y tienen en todo el mundo circulación más extensa que la de cualquiera otra publicación del mismo género. Las publica el Instituto Wistar de Anatomía y Biología, de Filadelfia, Estados Unidos de América, como obra cooperativa emprendida por el Instituto para estimular y ayudar las investigaciones biológicas, y están escritas por prominentes investigadores que trabajan en otros laboratorios de los Estados Unidos y Canadá.

Para mayores informes dirigirse a

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As desgraçadas condições em que se encontra a maioria das nações europeias, obrigaram os seus sabios a desviarem-se ou a suspenderem parcial ou totalmente os seus trabalhos de investigação. Varios jornaes de anatomia e zoologia suspenderam a sua publicação enquanto outros se tornaram menos accesiveis aos investigadores, especialmente nos laboratorios americanos.

Com o fim de auxiliar o desenvolvimento das sciencias anatomicas e zoologicas e prestar aos sabios os elementos de que elles necessitam registando os progressos da sciencia, os directores dos seguintes jornaes:

Journal of Morphology
Journal of Comparative Neurology
American Journal of Anatomy
Anatomical Record and
Journal of Experimental Zoology

desejam iniciar, por este meio, um movimento afim de fazer chegar ás suas columnas os trabalhos de todos os laboratorios do mundo.

Artigos sobre Anatomia e Zoologia, que forem approvados pelos directores dos respectivos jornaes serão immediatamente publicados em inglês, francês, allemão, espanhól ou italiano.

As citadas publicações contém uma grande somma do trabalho de pesquisas dos sabios norteamdricanos sobre sciencias biologicas e tem maior circulação no mundo do que outros quaesquer jornaes da mesma especialidade. São publicados pelo Wistar Institute of Anatomy and Biology de Philadelphia, E. U. A., como trabalho de cooperação do Instituto, com o fim de estimular e auxiliar as pesquisas e descobertas no campo da Biologia.

Os directores dos nossos jornaes scientificos são todos notaveis investigadores que trabalham em laboratorios dos Estados Unidos e do Canadá.

Para mais completos esclarecimentos os interessados devem dirigir-se ao

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THE ECONOMY OF COÖPERATION

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